

**No. 2022-1461**

---

In the United States Court of Appeals  
For the Federal Circuit

---

BAXALTA INCORPORATED, BAXALTA GMBH,  
*Plaintiffs - Appellants*

v.

GENENTECH, INC.,  
*Defendant - Appellee*

---

Appeal from the United States District Court for the District of Delaware  
No. 1:17-cv-00509, Hon. Timothy B. Dyk

---

**NONCONFIDENTIAL JOINT APPENDIX**

---

PAUL, WEISS, RIFKIND, WHARTON  
& GARRISON LLP

Eric Alan Stone  
Nicholas Groombridge  
Catherine Nyarady  
Jennifer Gordon  
Josephine Young  
Naz E. Wehrli

1285 Avenue of the Americas  
New York, NY 10019  
(212) 373-3000

*Counsel for Appellee*

MORGAN, LEWIS & BOCKIUS LLP

William R. Peterson  
1000 Louisiana St., Ste. 4000  
Houston, TX 77002  
(713) 890-5188

MORGAN, LEWIS & BOCKIUS LLP

Julie S. Goldemberg  
1701 Market Street  
Philadelphia, PA 19103  
(215) 963-5095

*Counsel for Appellants*

*Additional Counsel on Inside Cover*

---

PAUL, WEISS, RIFKIND, WHARTON  
& GARRISON LLP

Kenneth A. Gallo

David E. Cole

2001 K Street, NW

Washington, DC 20006-1047

(202) 223-7300

*Counsel for Appellee*

MORGAN, LEWIS & BOCKIUS LLP

Michael J. Abernathy

Christopher J. Betti

Maria E. Doukas

Karon N. Fowler

110 North Wacker Drive

Chicago, IL 60606

(312) 324-1000

*Counsel for Appellants*

**TABLE OF CONTENTS**

<b>ECF No.</b>	<b>Docket Text</b>	<b>Date Filed</b>	<b>Page No(s).</b>
573	[UNSEALED] MEMORANDUM OPINION. Signed by Judge Timothy Belcher Dyk on 1/13/2022. This order has been emailed to local counsel. (myr) (Entered: 01/13/2022)	1/13/2022	Appx30-76
574	ORDER granting 407 Genentech Inc.'s Motion for Summary Judgment. See the accompanying Memorandum Opinion for additional details. The parties shall meet and confer and propose any redactions to the Memorandum Opinion on or before January 18, 2022. Signed by Judge Timothy Belcher Dyk on 1/13/2022. (myr) (Entered: 01/13/2022)	1/13/2022	Appx77
	United States Patent No. US 7,033,590 B1	04/25/2006	Appx78-192
—	District Court Docket	Printed 4/26/2022	Appx193-241

**TABLE OF CONTENTS**  
(continued)

<b>ECF No.</b>	<b>Docket Text</b>	<b>Date Filed</b>	<b>Page No(s).</b>
1	COMPLAINT FOR PATENT INFRINGEMENT AND DECLARATORY JUDGMENT OF PATENT INFRINGEMENT - filed with Jury Demand against Chugai Pharmaceutical Co., Ltd., Genentech, Inc. – Magistrate Consent Notice to Pltf. ( Filing fee \$ 400, receipt number 0311-2134340.) - filed by Baxalta Incorporated, Baxalta GmbH. (Attachments: # 1 Exhibit A, # 2 Exhibit B, # 3 Exhibit C, # 4 Exhibit D, # 5 Exhibit E, # 6 Exhibit F, # 7 Exhibit G, # 8 Exhibit H, # 9 Exhibit I, # 10 Exhibit J, # 11 Exhibit K, # 12 Exhibit L, # 13 Exhibit M, # 14 Civil Cover Sheet) (rwc) (Entered: 05/04/2017)	05/04/2017	Appx242, Appx394, Appx397
41	MOTION for Preliminary Injunction - filed by Baxalta GmbH, Baxalta Incorporated. (Connolly, Colm) (Entered: 12/14/2017)	12/14/2017	Appx3150-3151



**TABLE OF CONTENTS**  
(continued)

<b>ECF No.</b>	<b>Docket Text</b>	<b>Date Filed</b>	<b>Page No(s).</b>
47	DECLARATION of Krishnaswamy, Ph.D. re 42 MEMORANDUM in Support by Baxalta GmbH, Baxalta Incorporated. (Attachments: # 1 Exhibit A, # 2 Exhibit B, # 3 Exhibit C, # 4 Exhibit D, # 5 Exhibit E, # 6 Exhibit F, # 7 Exhibit H, # 8 Exhibit I, # 9 Exhibit K, # 10 Exhibit L, # 11 Exhibit M, # 12 Exhibit N, # 13 Exhibit O, # 14 Exhibit P, # 15 Exhibit Q, # 16 Exhibit R, # 17 Exhibit S, # 18 Exhibit T, # 19 Exhibit U, # 20 Exhibit V, # 21 Exhibit W, # 22 Exhibit X, # 23 Exhibit Y, # 24 Exhibit Z, # 25 Exhibit AA) (Connolly, Colm) Modified on 12/15/2017 (mdb). (Entered: 12/14/2017)	12/14/2017	Appx4343-4346

**TABLE OF CONTENTS**  
(continued)

<b>ECF No.</b>	<b>Docket Text</b>	<b>Date Filed</b>	<b>Page No(s).</b>
48	DECLARATION of Anthony A. Kossiakoff, Ph.D. re 42 MEMORANDUM in Support by Baxalta GmbH, Baxalta Incorporated. (Attachments: # 1 Exhibit A, # 2 Exhibit B, # 3 Exhibit C, # 4 Exhibit D, # 5 Exhibit E, # 6 Exhibit F, # 7 Exhibit G, # 8 Exhibit H, # 9 Exhibit I, # 10 Exhibit J, # 11 Exhibit K, # 12 Exhibit L, # 13 Exhibit M, # 14 Exhibit N, # 15 Exhibit O, # 16 Exhibit P, # 17 Exhibit Q, # 18 Exhibit R, # 19 Exhibit S, # 20 Exhibit T, # 21 Exhibit U, # 22 Exhibit V, # 23 Exhibit W, # 24 Exhibit X, # 25 Exhibit Y, # 26 Exhibit Z, # 27 Exhibit AA, # 28 Exhibit BB, # 29 Exhibit CC, # 30 Exhibit DD, # 31 Exhibit EE, # 32 Exhibit FF, # 33 Exhibit GG, # 34 Exhibit HH) (Connolly, Colm) Modified on 12/15/2017 (mdb). (Entered: 12/14/2017)	12/14/2017	Appx5100, Appx5436-5437

**TABLE OF CONTENTS**  
(continued)

<b>ECF No.</b>	<b>Docket Text</b>	<b>Date Filed</b>	<b>Page No(s).</b>
111	<p>[SEALED] DECLARATION of John P. Sheehan, M.D. in Support of Genentech's Opposition to Baxalta's Motion for a Preliminary Injunction by Genentech, Inc. (Attachments: # 1 Exhibit A, # 2 Exhibit B, # 3 Exhibit C, # 4 Exhibit D, # 5 Exhibit E, # 6 Exhibit F, # 7 Exhibit G, # 8 Exhibit H, # 9 Exhibit I, # 10 Exhibit J, # 11 Exhibit K, # 12 Exhibit L, # 13 Exhibit M, # 14 Exhibit N, # 15 Exhibit O, # 16 Exhibit P, # 17 Exhibit Q, # 18 Exhibit R, # 19 Exhibit S, # 20 Exhibit T, # 21 Exhibit U, # 22 Exhibit V, # 23 Exhibit W, # 24 Exhibit X, # 25 Exhibit Y, # 26 Exhibit Z, # 27 Exhibit AA, # 28 Exhibit BB, # 29 Exhibit CC, # 30 Exhibit DD, # 31 Exhibit EE, # 32 Exhibit FF, # 33 Exhibit GG, # 34 Exhibit HH, # 35 Exhibit II, # 36 Exhibit JJ, # 37 Exhibit KK) (Mayo, Andrew) (Entered: 03/30/2018)</p>	03/30/2018	Appx6253, Appx6606

**TABLE OF CONTENTS**  
(continued)

<b>ECF No.</b>	<b>Docket Text</b>	<b>Date Filed</b>	<b>Page No(s).</b>
129	EXHIBIT re 128 Declaration Exhibit 1 by Baxalta GmbH, Baxalta Incorporated. (Attachments: # 1 Exhibit 2, # 2 Exhibit 3, # 3 Exhibit 4, # 4 Exhibit 6, # 5 Exhibit 8) (Dudash, Amy) (Entered: 04/11/2018)	04/11/2018	Appx9931-9999
158	CLAIM CONSTRUCTION OPENING BRIEF filed by Baxalta GmbH, Baxalta Incorporated. (Dudash, Amy) (Entered: 05/18/2018)	05/18/2018	Appx10620, Appx10641-10644
163	[SEALED] DECLARATION re 160 Claim Construction Opening Brief (Declaration of John P. Sheehan, M.D.) by Genentech, Inc. (Attachments: # 1 Exhibit A-H) (Balick, Steven) (Entered: 05/18/2018)	05/18/2018	Appx11236, Appx11434
262	OPINION & ORDER denying 41 MOTION for Preliminary Injunction Signed by Judge Timothy Belcher Dyk on 8/7/2018. (nmf) (Entered: 08/07/2018)	08/07/2018	Appx16003

**TABLE OF CONTENTS**  
(continued)

<b>ECF No.</b>	<b>Docket Text</b>	<b>Date Filed</b>	<b>Page No(s).</b>
275	ORDER, the documents listed in Exhibit A to the parties' joint letter, ECF No. 245-1, are ADMITTED and are part of the preliminary injunction record. The parties' objections to inclusion of these documents are OVERRULED. Signed by Judge Timothy Belcher Dyk on 8/10/2018. (crb) (Entered: 08/10/2018)	08/10/2018	Appx16032
293	SO ORDERED, 292 First Amended Stipulation and Order Dismissing Defendant Chugai Pharmaceutical Co., Ltd. Signed by Judge Timothy Belcher Dyk on 9/19/2018. (ceg) (Entered: 09/19/2018)	09/19/2018	Appx16033
330	OPINION AND ORDER. Signed by Judge Timothy Belcher Dyk on 12/3/2018. (nmg) (Entered: 12/03/2018)	12/03/2018	Appx16160-16190
335	STIPULATION AND FINAL JUDGMENT by Baxalta GmbH, Baxalta Incorporated. (Dudash, Amy) (Entered: 01/31/2019)	1/31/2019	Appx16195-16197

**TABLE OF CONTENTS**  
(continued)

<b>ECF No.</b>	<b>Docket Text</b>	<b>Date Filed</b>	<b>Page No(s).</b>
337	FINAL JUDGMENT is entered in favor of Genentech, Inc. and against Baxalta Incorporated on all claims asserted in the operative complaint. Genetech's counterclaims for declaratory judgment of non-infringement and a declaratory judgment of invalidity are dismissed without prejudice (*see Order for further details) (*CASE CLOSED). Signed by Judge Timothy Belcher Dyk on 1/31/2019. (ceg) (Entered: 02/01/2019)	02/01/2019	Appx16199-16202
407	MOTION for Summary Judgment - filed by Genentech, Inc.. (Attachments: # 1 Text of Proposed Order) (Balick, Steven) (Entered: 09/03/2021)	9/3/2021	Appx16211
408	[SEALED] OPENING BRIEF in Support re 407 MOTION for Summary Judgment filed by Genentech, Inc. Answering Brief/Response due date per Local Rules is 9/17/2021. (Balick, Steven) (Entered:09/03/2021)	9/3/2021	Appx16215-16219, Appx16230, Appx16242, Appx16249-16252

**TABLE OF CONTENTS**  
(continued)

<b>ECF No.</b>	<b>Docket Text</b>	<b>Date Filed</b>	<b>Page No(s).</b>
409	[SEALED] DECLARATION re 408 Opening Brief in Support (Declaration of David E. Cole, Volume 1 of 2) by Genentech, Inc.. (Attachments: # 1 Exhibit 1-14 (Volume 1 of 2)) (Balick, Steven) (Entered:09/03/2021)	9/3/2021	Appx16261, Appx16399, Appx16413-16422, Appx16431-16440, Appx16448-16452, Appx16514-16651, Appx16699, Appx16818, Appx16828-16892, Appx16971, Appx16974-16975, Appx16991-16994
410	[SEALED] DECLARATION re 408 Opening Brief in Support (Declaration of David E. Cole, Volume 2 of 2) by Genentech, Inc.. (Attachments: # 1 Exhibit 15-28 (Volume 2 of 2)) (Balick, Steven) (Entered: 09/03/2021)	9/3/2021	Appx17275, Appx17279-17280, Appx17298, Appx17313-17315, Appx17339-17341, Appx17350-17353, Appx17411, Appx17435-17436, Appx17451-17453, Appx17466-17468, Appx17599-17600, Appx17607-17608, Appx17668-17671, Appx17684, Appx17711-17712

**TABLE OF CONTENTS**  
(continued)

<b>ECF No.</b>	<b>Docket Text</b>	<b>Date Filed</b>	<b>Page No(s).</b>
413	[SEALED] DECLARATION re 408 Opening Brief in Support (Declaration of William R. Strohl, Ph.D.) by Genentech, Inc.. (Attachments: # 1 Exhibit 1-3) (Balick, Steven) (Entered: 09/03/2021)	9/3/2021	Appx18164, Appx18184-18186, Appx18202-18207, Appx18215-18217, Appx18248-18254, Appx18418-18426
415	[SEALED] DECLARATION re 408 Opening Brief in Support (Declaration of Dr. K. Christopher Garcia) by Genentech, Inc.. (Attachments: # 1 Exhibit 1-2) (Balick, Steven) (Entered: 09/03/2021)	9/3/2021	Appx18575, Appx18615-18628, Appx18645, Appx18754-18755



**TABLE OF CONTENTS**  
(continued)

<b>ECF No.</b>	<b>Docket Text</b>	<b>Date Filed</b>	<b>Page No(s).</b>
424	[SEALED] ANSWERING BRIEF in Opposition re 407 MOTION for Summary Judgment filed by Baxalta GmbH, Baxalta Incorporated. Reply Brief due date per Local Rules is 10/8/2021. (Attachments: # 1 Index of Exhibits, # 2 Exhibit 1, # 3 Exhibit 2, # 4 Exhibit 3, # 5 Exhibit 4, # 6 Exhibit 5, # 7 Exhibit 6, # 8 Exhibit 7, # 9 Exhibit 8, # 10 Exhibit 9, # 11 Exhibit 10, # 12 Exhibit 11, # 13 Exhibit 12, # 14 Exhibit 13, # 15 Exhibit 14, # 16 Exhibit 15, # 17 Exhibit 16, # 18 Exhibit 17, # 19 Exhibit 18, # 20 Exhibit 19, # 21 Exhibit 20, # 22 Exhibit 21, # 23 Exhibit 22, # 24 Exhibit 23, # 25 Exhibit 24, # 26 Exhibit 25, # 27 Exhibit 26, # 28 Exhibit 27, # 29 Exhibit 28, # 30 Certificate of Service) (Dudash, Amy) (Entered: 10/01/2021)	10/1/2021	Appx18836, Appx18843-18856, Appx18861-18875, Appx19021-19023, Appx19121-19122, Appx19132-19135, Appx19141-19142, Appx19147-19148, Appx19151, Appx19163, Appx19176, Appx19179, Appx19186-19192, Appx19197-19198, Appx19205-19226, Appx19256, Appx19321-19322, Appx19337, Appx19387-19398, Appx19519-19520, Appx19524-19525, Appx19530-19533, Appx19539-19545, Appx19670-19723, Appx19776, Appx19807
425	[SEALED] REPLY BRIEF re 407 MOTION for Summary Judgment filed by Genentech, Inc.. (Balick, Steven) (Entered: 10/15/2021)	10/15/2021	Appx20329, Appx20335

**TABLE OF CONTENTS**  
(continued)

<b>ECF No.</b>	<b>Docket Text</b>	<b>Date Filed</b>	<b>Page No(s).</b>
431	Official Transcript of Hearing held on 11/19/2021 before Judge Timothy B. Dyk. Transcript may be viewed at the court public terminal or order/purchased through the Court Reporter/Transcriber before the deadline for Release of Transcript Restriction. After that date, it may be obtained through PACER. Redaction Request due 12/15/2021. Redacted Transcript Deadline set for 12/27/2021. Release of Transcript Restriction set for 2/22/2022. (cna, ) (Entered: 11/29/2021)	11/24/2021	Appx20356, Appx20363-20365, Appx20402-20411, Appx20432-20435
437	STIPULATION of Fact Regarding Hybridoma Technology and the Number of Anti-Factor IX/IXa Antibodies Disclosed In The '590 Patent, by Genentech, Inc.. (Balick, Steven) (Entered: 12/03/2021)	12/3/2021	Appx20569-20572
578	NOTICE OF APPEAL to the Federal Circuit of 574 Order on Motion for Summary Judgment, 573 Memorandum Opinion. Appeal filed by Baxalta GmbH, Baxalta Incorporated. (Dudash, Amy) (Entered: 02/08/2022)	2/8/2022	Appx20575-20576

**TABLE OF CONTENTS**  
(continued)

<b>ECF No.</b>	<b>Docket Text</b>	<b>Date Filed</b>	<b>Page No(s).</b>
—	Baxalta's Summary Judgment Hearing Hand-up	11/19/2021	Appx20615-20620

**STATEMENT OF CONFIDENTIALITY**

Materials omitted from the following of the nonconfidential version of the Joint Appendix were filed under seal or redacted below, pursuant to the protective order filed with the District Court on October 29, 2018 (Dkt. 316): Appx6253, Appx6606, Appx11236, Appx11434, Appx16215-16219, Appx16230, Appx16242, Appx16249-16252, Appx16261, Appx16399, Appx16413-16422, Appx16431-16440, Appx16448-16452, Appx16514-16651, Appx16699, Appx16818, Appx16828-16892, Appx16971, Appx16974-16975, Appx16991-16994, Appx17275, Appx17279-17280, Appx17298, Appx17313-17315, Appx17339-17341, Appx17350-17353, Appx17411, Appx17435-17436, Appx17451-17453, Appx17466-17468, Appx17599-17600, Appx17607-17608, Appx17668-17671, Appx17684, Appx17711-17712, Appx18164, Appx18184-18186, Appx18202-18207, Appx18215-18217, Appx18248-18254, Appx18418-18426, Appx18575, Appx18615-18628, Appx18645, Appx18754-18755, Appx18836, Appx18843-18856, Appx18861-18875, Appx19021-19023, Appx19121-19122, Appx19132-

**TABLE OF CONTENTS**  
(continued)

19135, Appx19141-19142, Appx19147-19148, Appx19151, Appx19163, Appx19176, Appx19179, Appx19186-19192, Appx19197-19198, Appx19205-19226, Appx19256, Appx19321-19322, Appx19337, Appx19387-19398, Appx19519-19520, Appx19524-19525, Appx19530-19533, Appx19539-19545, Appx19670-19723, Appx19776, Appx19807, Appx20329, and Appx20335. This material consists of proprietary business information not available to the public.

**IN THE UNITED STATES DISTRICT COURT  
FOR THE DISTRICT OF DELAWARE**

BAXALTA INCORPORATED and  
BAXALTA GMBH,

Plaintiffs,

v.

GENENTECH, INC. and CHUGAI  
PHARMACEUTICAL CO., LTD.,

Defendants.

Civil Action No. 17-509-TBD

**MEMORANDUM OPINION**

Pending before the court is Genentech’s motion for summary judgment. Genentech moves for summary judgment of 1) invalidity of claims 1–4, 19, and 20 of the ’590 patent for lack of written description and enablement, 2) non-infringement under a doctrine-of-equivalents theory, and 3) no willful infringement. For the reasons stated below, the court GRANTS Genentech’s motion for summary judgment of invalidity for lack of enablement and need not address Genentech’s motion in all other respects.

**I. PROCEDURAL HISTORY**

On May 4, 2017, Baxalta Inc. and Baxalta GmbH (together, “Baxalta”) brought suit against Genentech, Inc. and Chugai Pharmaceutical Co., Ltd., alleging infringement of U.S. Patent No. 7,033,590 (“the ’590 patent”) by the manufacture, use, sale, offer to sell, and importation of an antibody used to treat hemophilia A and known as emicizumab or ACE910, marketed under the brand name Hemlibra. Compl., ECF No. 1, ¶¶ 37–51. Chugai was subsequently dismissed from

the case.<sup>1</sup> Genentech answered on June 30, denying Baxalta's allegations and counterclaiming for declaratory judgment of noninfringement and invalidity on grounds of lack of enablement and written description support. Answer & Countercl., ECF No. 9, ¶¶ 37–51, 120–49.

On December 14, 2017, Baxalta moved for a preliminary injunction against Genentech. *See* Mot. for Prelim. Inj., ECF No. 41. On August 7, 2018, the court denied Baxalta's motion, finding that it had not proven a likelihood of success with respect to infringement and invalidity, and that even if it had, "given the ample evidence of medical need, the public interest weigh[ed] strongly against issuing a preliminary injunction since Hemlibra has unique medical benefits not available from Baxalta's competing products." Prelim. Inj. Order, ECF No. 262, at 24; *id.* at 28–29.

On December 3, 2018, following a Markman hearing, the court issued a claim construction decision in which it construed the term "antibody" to exclude bispecific antibodies. *See* Claim Construction Order, ECF No. 330, at 22–23. Thereafter, the parties stipulated to non-infringement of the asserted claims under the court's claim construction. *See* Stipulations, ECF Nos. 331–332. The court entered judgment in Genentech's favor on February 1, 2019. *See* Stip. & Final J., ECF No. 337. Baxalta appealed, and on August 27, 2020, the Federal Circuit issued a decision rejecting this court's construction of the terms "antibody" and "antibody fragment," determining that the term antibody included bispecific antibodies, and vacating the judgment of non-infringement and remanding for further proceedings. *See Baxalta Inc. v. Genentech, Inc.*, 972 F.3d 1341, 1343, 1349 (Fed. Cir. 2020) (construing antibody to mean "an immunoglobulin molecule having a

---

<sup>1</sup> Chugai is a Japanese company that invented and manufactures the accused product, Hemlibra, in Japan. *See, e.g.,* Yamaguchi Decl., ECF No. 20, ¶¶ 2, 5. Hemlibra is shipped to the United States where it is sold by Genentech. *See id.* ¶¶ 7, 10. The parties stipulated to the dismissal of Chugai as a defendant in this case in June 2018. *See* Stip. & Prop. Order, ECF No. 220; July 2, 2018, Min. Entry.

specific amino acid sequence comprising two heavy chains (H chains) and two light chains (L chains)” and “antibody fragment” to mean “a portion of an antibody”). Upon remand, the case proceeded with fact and expert discovery.

On September 3, 2021, Genentech filed a motion for summary judgment of 1) invalidity of claims 1–4, 19, and 20 of the ’590 patent for lack of written description and enablement, 2) non-infringement under a doctrine-of-equivalents theory, and 3) no willful infringement. *See* Opening Br. in Supp. of Genentech’s Mot. for Summ. J., ECF No. 416 (Def.’s Mot.), at 1, 15–16. Baxalta thereafter filed its opposition to Genentech’s motion, *see* Pl.’s Opp’n to Def.’s Mot. for Summ. J., ECF No. 424 (Pl.’s Opp’n), and Genentech filed its reply on October 15, 2021, *see* Reply Br. in Supp. of Genentech, Inc.’s Mot. for Summ. J., ECF No. 425. The parties have submitted expert declarations and exhibits, as well as a Joint Stipulation of Fact. *See* Joint Stip. of Fact Regarding Hybridoma Tech. & the Number of Anti-Factor IX/IXa Antibodies Disclosed in the ’590 Patent, ECF No. 437 (Joint Stip.). The court heard oral argument on the motion on November 19, 2021. *See* Nov. 22, 2021, Min. Entry.

## II. SUMMARY OF DECISION

For the reasons described in detail below, the court finds that Genentech has shown by clear and convincing evidence that the asserted claims of the ’590 patent are not enabled. There are millions of candidate antibodies within the genus and a dearth of working examples of those that satisfy the claim limitations. There are only eleven working examples disclosed in the patent. The examples are all murine, monospecific antibodies of the IgG and IgM isotypes, or fragments thereof. The genus of independent claim 1 is functionally and structurally broad. And in many respects there are no examples in the specification for the covered classes of antibodies. For example:

1. Claim 1 covers an antibody that increases the procoagulant activity of Factor IXa by an amount ranging from barely perceptible to an amount capable of use in “a preparation for the treatment of blood coagulation disorders which has particular advantages for factor VIII inhibitor patients.” ’590 patent, col. 2, ll. 25–28. Claim 1 thus covers an antibody or antibody fragment that increases the procoagulant activity of Factor IXa in the presence of Factor VIII inhibitors.<sup>2</sup> There is no working example of an antibody that increases the procoagulant activity of Factor IXa by more than a marginal amount in the presence of Factor VIII inhibitors. And for the non-inhibitor population, there is no working example of an antibody that increases the procoagulant activity of Factor IXa by an amount capable of moving a patient with a severe hemophilia A condition (comprising over 60% of hemophilia A patients) to a mild condition. Baxalta’s expert concedes that the patent’s assertions that antibodies of the invention have therapeutic utility is merely “aspirational.”
2. Claim 1 covers humanized and chimeric antibodies. There are no working examples of humanized or chimeric antibodies disclosed in the specification.
3. Claim 1 covers bispecific antibodies such as the accused product emicizumab. There are no working examples of bispecific antibodies disclosed in the specification.
4. Claim 1 covers antibodies of the IgE isotype. There are no working examples of IgE antibodies disclosed in the specification.

---

<sup>2</sup> As discussed below, an “inhibitor patient” is someone who has developed an immune response to traditional Factor VIII replacement therapies.



5. Claim 1 covers antibodies of the IgA isotype. There are no working examples of IgA antibodies disclosed in the specification.
6. Claim 1 covers antibodies of the IgD isotype. There are no working examples of IgD antibodies disclosed in the specification.
7. Claim 1 covers diabodies and dimers, oligomers, and multimers of the claimed antibodies. There are no working examples of diabodies or dimers, oligomers, or multimers of antibodies in the specification.

The specification also provides no guidance as to how to identify which antibodies will satisfy the claim limitations, nor does it describe what structural or other features of the disclosed antibodies cause them to bind to Factor IX/IXa or to increase the procoagulant activity of Factor IXa. The field of antibodies is inherently unpredictable. The only way to practice the teachings of the patent is by trial-and-error; *i.e.*, by screening tens of thousands, if not millions, of candidate antibodies to determine whether they satisfy the limitations of the asserted claims.

The same deficiencies exist as to dependent claims 3–4, 19, and 20, which include the same functional limitations as claim 1 but also specify structural limitations.

This is not adequate enablement under Federal Circuit precedent, including *In re Wands*, 858 F.2d 731, 737 (Fed. Cir. 1988), and the cases that have followed, because it requires undue experimentation to practice the full scope of what is claimed.

### III. LEGAL STANDARD

#### A. Summary Judgment

Under Rule 56(a) of the Federal Rules of Civil Procedure, “[t]he court shall grant summary judgment if the movant shows that there is no genuine dispute as to any material fact and the movant is entitled to judgment as a matter of law.” The moving party bears the burden of

demonstrating the absence of a genuine issue of material fact. *See Celotex Corp. v. Catrett*, 477 U.S. 317, 323 (1986). If the moving party has carried its burden, the nonmovant must then “come forward with ‘specific facts showing that there is a *genuine issue for trial*.’” *Matsushita Elec. Indus. Co. v. Zenith Radio Corp.*, 475 U.S. 574, 587 (1986) (quoting Fed. R. Civ. P. 56(e)). The court “must draw all reasonable inferences in favor of the nonmoving party, and it may not make credibility determinations or weigh the evidence.” *Reeves v. Sanderson Plumbing Prods., Inc.*, 530 U.S. 133, 150 (2000).

B. 35 U.S.C. § 112

One of the statutory conditions for patentability under the Patent Act is adequate disclosure of the invention. Section 112 provides, in pertinent part, that:

The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same, and shall set forth the best mode contemplated by the inventor of carrying out his invention.

35 U.S.C. § 112. Section 112 imposes two separate requirements. The first is the written description requirement, found in the first sentence of Section 112, which requires that the specification contain an adequate “written description of the invention.” 35 U.S.C. § 112; *see also Ariad Pharms., Inc. v. Eli Lilly & Co.*, 598 F.3d 1336, 1346 (Fed. Cir. 2010) (en banc) (“[A] separate requirement to describe one’s invention is basic to patent law . . . It is part of the *quid pro quo* of a patent; one describes an invention, and, if the law’s other requirements are met, one obtains a patent.”). The inquiry into written description is a question of fact but it is “amenable to summary judgment in cases where no reasonable fact finder could return a verdict for the non-

moving party.” *Bos. Sci. Corp. v. Johnson & Johnson*, 647 F.3d 1353, 1361 (Fed. Cir. 2011) (internal quotation marks and citation omitted).

The second requirement is enablement. “Whether a claim satisfies the enablement requirement of 35 U.S.C. § 112 is a question of law.” *Amgen Inc. v. Sanofi, Aventisub LLC*, 987 F.3d 1080, 1084 (Fed. Cir. 2021). An enabling disclosure is the “*quid pro quo* of the right to exclude.” *J.E.M. Ag Supply, Inc. v. Pioneer Hi-Bred Intern., Inc.*, 534 U.S. 124, 142 (2001). To be enabling, “the specification of a patent must teach those skilled in the art how to make and use the full scope of the claimed invention without undue experimentation.” *Genentech, Inc. v. Novo Nordisk A/S*, 108 F.3d 1361, 1365 (Fed. Cir. 1997) (quoting *In re Wright*, 999 F.2d 1557, 1561 (Fed. Cir. 1993)); see *In re Fisher*, 427 F.2d 833, 839 (C.C.P.A. 1970) (“[T]he scope of the claims must bear a reasonable correlation to the scope of enablement provided by the specification to persons of ordinary skill in the art.”). Although it is not necessary to disclose every species within a genus, see *In re Angstadt*, 537 F.2d 498, 502–03 (C.C.P.A. 1976), “there must be sufficient disclosure, either through illustrative examples or terminology, to teach those of ordinary skill how to make and how to use the invention as broadly as it is claimed,” *In re Vaeck*, 947 F.2d 488, 496 (Fed. Cir. 1991).

To be valid, a patent must satisfy both the written description and enablement requirements. See *Ariad*, 598 F.3d at 1351. Because, for the reasons discussed below, the court finds that no reasonable jury could find the full scope of the asserted claims of the ’590 patent are enabled, it need not separately address written description, though the claims may also be invalid for lack of written description support.

#### IV. FACTUAL BACKGROUND

The '590 patent is directed to an antibody or antibody derivative that binds to a protein important for blood coagulation known as Factor IX (or Factor IXa) and increases the procoagulant activity of Factor IXa, for use in treatment of hemophilia A patients, particularly those who have developed Factor VIII inhibitors. '590 patent, col. 2, ll. 25–33. Asserted here are independent claim 1 and dependent claims 2–4, 19 and 20, which recite:

1. An isolated antibody or antibody fragment thereof that binds Factor IX or Factor IXa and increases the procoagulant activity of Factor IXa.
2. The antibody or antibody fragment according to claim 1 that increases the procoagulant activity of Factor IXa in the presence of Factor VIII inhibitors.
3. The antibody or antibody fragment according to claim 1 wherein the antibody is an IgG, IgM, IgA or IgE antibody.
4. The antibody or antibody fragment according to claim 1, wherein said antibody or antibody fragment is selected from the group consisting of a monoclonal antibody, a chimeric antibody, a humanized antibody, a single chain antibody, a bispecific antibody, a diabody, and di-, oligo- or multimers thereof.
19. The antibody or antibody fragment according to claim 4, wherein the antibody is a humanized antibody.
20. The antibody or antibody fragment according to claim 2, wherein the antibody is selected from the group consisting of an IgG, IgM, IgA, or IgE antibody.

Some background on antibodies and hemophilia A as well as its prior-art treatment based on the parties' agreed-upon views is necessary.

##### A. Hemophilia A and Its Prior-Art Treatment

The body stops bleeding by relying on blood coagulation, also known as clotting, which is accomplished through a cascade of reactions between proteins. *See* Sheehan Decl., ECF No. 411,

Ex. 1, Opening Rpt. ¶¶ 23–24 (Sheehan Rpt.); Pl.’s Opp’n, Ex. 8, ECF No. 424-9, Krishnaswamy Rebuttal Rpt. ¶ 25 (Krishnaswamy Rpt.). The individual coagulation proteins are referred to as coagulation “Factors,” with respective assigned Roman numerals (*e.g.*, Factor VIII and Factor IX). Sheehan Rpt. ¶ 23; Krishnaswamy Rpt. ¶ 25. These Factors normally circulate in the blood in inactive forms until triggered by a vascular injury, which causes a coagulation cascade. *See* Sheehan Rpt. ¶ 23. Factors in their activated form are identified with an appended “a” (*e.g.*, Factor IXa). *See id.*; Opp’n Br., Ex. 2, Malackowski Opening Rpt. (Malackowski Rpt.), at 20. The relevant steps in the clotting cascade for present purposes are the coming together of Factor VIIIa and Factor IXa. *See* Sheehan Rpt. ¶ 25; Krishnaswamy Rpt. ¶ 26. In a healthy person, activated Factor VIII (Factor VIIIa) “complexes with” activated Factor IX (Factor IXa) and Factor X, causing Factors IXa to activate Factor X to Factor Xa, which is essential for clot formation. *See* Sheehan Rpt. ¶ 25; Krishnaswamy Rpt. ¶ 26.

Hemophilia A is a genetic disorder in which patients lack sufficient functional Factor VIII. Young Decl., ECF No. 414, Rebuttal Report ¶ 14 (Young Decl.); Krishnaswamy Rpt. ¶ 27. This amounts to a roadblock in the clotting cascade, and hemophilia A patients therefore suffer from a reduced ability to form quick and effective blood clots. Without Factor VIII, and without treatment, hemophilia A patients are at risk of bleeding episodes not only from external trauma, but internally into joints and other spaces in the body. Young Decl. ¶ 14. Hemophilia A can be classified as mild, moderate, or severe, depending on the relative level of Factor VIII present. Sheehan Rpt. ¶¶ 31–32; Malackowski Rpt. at 21. There are approximately 23,000–25,000 males with hemophilia A living in the United States. Young Decl. ¶ 16. About half of them have been diagnosed with a severe form of the disorder. *Id.* Females are less likely to have severe

hemophilia A because the genetic mutation associated with hemophilia A is “X-linked recessive.”

Young Decl. ¶ 15.

Historically, the only treatment for hemophilia A patients was infusion (intravenous) with a Factor VIII replacement, either as needed when bleeding episodes occur (on-demand) or in a preventative matter (prophylaxis). *Id.* ¶ 17; Krishnaswamy Rpt. ¶ 28. The problem with that treatment, however, was that 25–30% of patients with severe hemophilia who were treated with Factor VIII replacement therapies developed an immune response to Factor VIII. Young Decl. ¶ 22; Malackowski Rpt. at 21. This immune response is known as an “inhibitor,” and the patients exhibiting this response are known as the “inhibitor population.” Young Decl. ¶ 22. As a result, Factor VIII replacement therapy is usually not effective in the inhibitor population. *Id.* ¶ 23; Krishnaswamy Rpt. ¶ 29.

Before the introduction of Hemlibra, the inhibitor population had few effective treatment options. *See* Young Decl. ¶¶ 22–27; Malackowski Rpt. at 21. One option was a therapy called “Immune Tolerance Induction (ITI).” Young Decl. ¶ 24; Malackowski Rpt. at 21. But that treatment is costly, complicated, and prolonged, requiring daily intravenous infusions of high concentrations of Factor VIII over the course of months or even years until the body’s immune system begins to tolerate it, if ever. *See* Young Decl. ¶ 24; Malackowski Rpt. at 21.

As of 2018, inhibitor patients could also take one of two “bypass agents” (BPAs), including Baxalta’s product FEIBA (“Factor Eight Inhibitor Bypassing Activity”). *See* Young Decl. ¶ 25; Malackowski Rpt. at 21. BPAs work by bypassing the Factor VIII step in the clotting cascade. *See* Young Decl. ¶ 25; Malackowski Rpt. at 21. Like Factor VIII replacement therapy, BPAs can be used in two ways: on-demand when a bleeding episode occurs and/or on a regular schedule as prophylaxis. Young Decl. ¶ 26; Malackowski Rpt. at 25. But they too must be infused, which

may impose a substantial treatment burden on patients and their families. In particular, the infusion can take up to an hour as often as every other day in order to achieve the desired prophylactic effect. *See* Young Decl. ¶ 27; Malackowski Rpt. at 34.

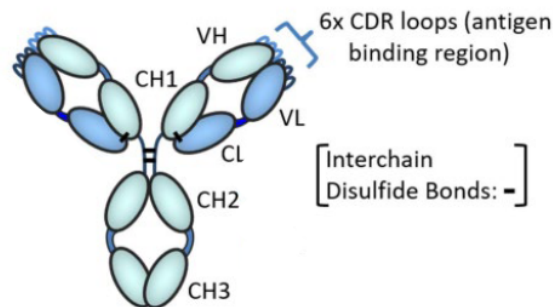
#### B. Baxalta's Search for an Antibody-Based Hemophilia A Treatment

Recognizing the drawbacks with all the existing treatment options, in 1998, scientists working for Baxalta's predecessor began experimenting with ideas for new, better hemophilia A treatments, particularly for inhibitor patients. Dr. Friedrich Scheiflinger, one of the '590 patent's named inventors, had the idea of using an antibody against Factor IX/IXa to increase the procoagulant activity of Factor IXa even in the absence of Factor VIII. *See* '590 patent, col. 2, ll. 29–44; Cole Decl. vol. 1, Ex. 11, Scheiflinger Dep. Tr. at 98:8–99:20, 101:02–12.

##### 1. Antibody Structure and Genetic Modification

Antibodies are a key component of the immune system. Strohl Decl., ECF No. 413, Ex. 1, Opening Rpt., ¶ 35 (Strohl Rpt.). When confronted with a foreign molecule, or “antigen,” the immune system's “B cells” (a type of white blood cell) generate antibodies that attack the antigen by binding to them. *See id.* ¶¶ 35, 41; Garcia Decl., ECF No. 415, Ex. 1, ECF No. 415-1, Opening Rpt. (Garcia Rpt.), ¶ 58. Each unique B-cell produces multiple copies of one specific antibody—meaning, the secreted antibody can bind to only one antigen. Garcia Rpt. ¶ 59. The binding of an antigen to the B-cell surface stimulates the B-cell to divide and mature into identical cells, secreting millions of antibodies into the bloodstream and lymphatic system. *See id.* ¶ 57. An antibody, as that term has been construed in the '590 patent, is “an immunoglobulin molecule having a specific amino acid sequence comprising two heavy chains (H chains) and two light chains (L chains).” *Baxalta*, 972 F.3d at 1349.

Antibodies can be visualized as forming a “Y” shape, with two arms connected by disulfide bonds. Strohl Rpt. ¶ 35. Each arm of the Y shape contains two polypeptides known as the heavy (H) chain and the light (L) chain. *See id.* Each of its heavy and light chains consist of two regions. *See id.* ¶ 36. The portions of the heavy and light chains that vary from antibody to antibody depending on the antigen are called the “variable domains,” designated VH and VL, respectively. *Id.* ¶¶ 36–37. Variable regions include (i) complementarity-determining regions (“CDRs”), which are amino acid sequences that play a key role in the antibody’s binding to an antigen, and (ii) framework regions, which serve as “scaffolds for the CDRs.” *Id.* ¶¶ 36, 39. The remaining portions are called Constant (C) regions of each chain. *See id.* ¶ 36. This is a schematic of an antibody:



*Id.* ¶ 35 (Figure 1).

Based on the structure of the constant regions, antibodies are grouped into five classes—IgA, IgD, IgE, IgG, and IgM—each with closely related but different functions. *Id.* ¶ 37.<sup>3</sup> The constant region of all antibodies of the same isotype are identical (*e.g.*, all IgG antibodies have the same constant region and that constant region differs from that of IgA antibodies). *Id.* Because

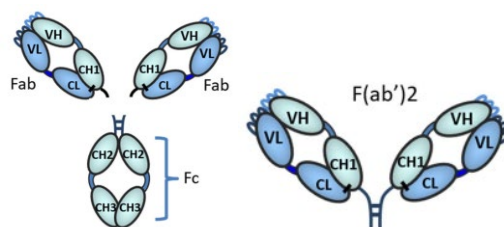
<sup>3</sup> “Ig” stands for immunoglobulin, and each letter signifies the specific class, which may change depending on the stage of the immune response. *See* Marasco Rpt. ¶ 71. The various antibody isotypes “differ from one another in biological properties, functional locations, and ability to deal with different antigens.” Marasco Rpt. ¶ 278. IgGs are the most prevalent class, whereas IgDs are the least prevalent. *See* Strohl Rpt. ¶ 37.



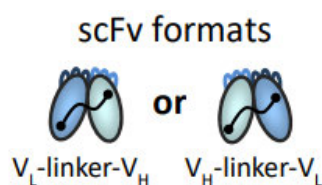
each arm of a naturally occurring antibody is identical, each arm targets the same antigen. *Id.* ¶ 38.

Naturally occurring antibodies are thus said to be “monospecific.” *Id.*

Scientists have developed various genetic engineering techniques for altering natural antibodies to make a wide variety of molecules. Some are of different sizes than natural antibodies, whereas others have different binding specificities or different constant-region functions. For example, scientists have used protein-cleaving enzymes to cut antibodies into “antibody fragments.” *Id.* ¶¶ 46–47. These include the Fab, Fc, and F(ab')<sub>2</sub> fragments, shown below.

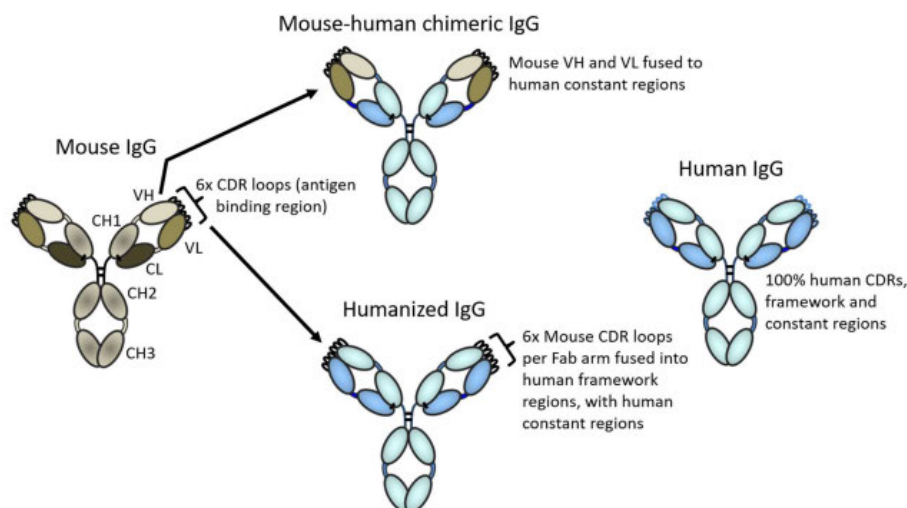


*Id.* at Figs. 2–3. They have also used recombinant DNA techniques to derive antibody fragments beyond simple enzymatic cleavage of a full-length antibody. One example (depicted below) is a fragment called a single-chain Fv (scFv), which contains the variable region of a heavy chain and the variable region of a light chain, held together by a synthetic string of amino acids. *Id.* ¶ 48.



*Id.* at Fig. 4.

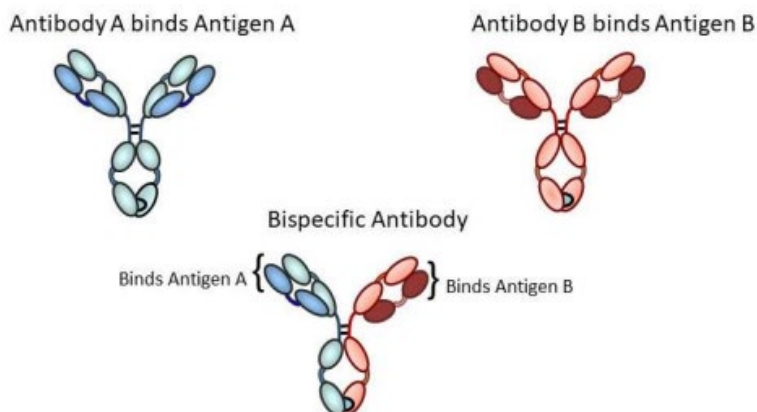
Genetic engineering has also made possible the construction of antibodies that are part animal sequence and part human sequence. *Id.* ¶ 49. These are known as “chimeric” and “humanized” antibodies—as shown below. *Id.*



*Id.* at Fig. 5. The benefit of humanizing antibodies is that it lessens the chances of an immune response to the antibody. *Id.* ¶ 50. In early efforts to use mouse (or murine) antibodies as therapeutic agents in humans, scientists observed that the human immune system recognized the murine antibody as a foreign substance and made antibodies against it. *Id.* This became known as the “HAMA response,” for “human anti-mouse antibody.” *Id.* It spurred research to develop a way to engineer antibodies containing more human amino acid sequences and less animal (*e.g.*, murine) sequences. *Id.* ¶ 51.

Initially, scientists used genetic engineering techniques to create “chimeric antibodies” by splicing together genetic material (DNA) encoding the variable regions of animal antibodies (usually murine) with DNA encoding the constant regions of human antibodies. *Id.* ¶¶ 52–53; ’590 patent, col. 6, l. 64–col. 7, l. 3. Although successful at first, over time it became clear that humans were developing a HAMA response to the murine sequences in the chimeric antibodies. Strohl Rpt. ¶ 54. To avoid that response, scientists designed “humanized antibodies” wherein non-human CDRs are inserted into an otherwise-human antibody. ’590 patent, col. 6, ll. 49–57. In the resulting antibody, the binding affinity is preserved, while adverse human immune reaction is significantly reduced as compared to the original animal antibody. Strohl Rpt. ¶¶ 55–56.

Finally, scientists have created “bispecific antibodies” by pairing the heavy and light chains of an antibody that binds to one antigen with the heavy and light chains of a different antibody that binds to a different antigen. *Id.* ¶ 61. The resulting antibody, depicted below, is thus capable of binding two antigens. *Id.*; ’590 patent, col. 7, ll. 32–34.



Strohl Rpt. at Fig. 7.

## 2. The ’590 Patent

Against this backdrop, in 1998, the scientists at Baxalta were experimenting with the idea of using an antibody binding to Factor IX/IXa to increase the procoagulant activity of Factor IXa even in the absence of Factor VIII. ’590 patent, col. 2, ll. 29–44. Over the course of approximately four years, they used hybridoma techniques (described below) to create monospecific antibodies that bind to Factor IX or IXa and increase the procoagulant activity of Factor IXa. Cole Decl. vol. 1, ECF No. 409 (“Cole Decl. vol. 1”), Ex. 13, Kerschbaumer Dep. Tr. at 14:325–15:328; ’590 patent, col. 7, l. 65–col. 8, l. 1; col. 9, l. 66–col. 10, l. 37; Sheehan Rpt. ¶ 166.

The ’590 patent, titled “Factor IX/factor IXa activating antibodies and antibody derivatives,” was filed on September 14, 2000, issued on April 25, 2006, and expired in December 2021. *See* Def.’s Mot. at 10. In total, the ’590 patent discloses eleven working examples of

antibodies that bind to Factor IX/IXa and increase the procoagulant activity of Factor IXa.<sup>4</sup> Pl.’s Opp’n, Ex. 4, Marasco Rebuttal Rpt., ECF No. 424-5, ¶ 124 (Marasco Rpt.), ¶ 111. To find the eleven examples, the inventors tested tens of thousands of antibodies in assays designed to measure Factor VIII-like activity. *See* ’590 patent, col. 10, l. 39–col. 12, l. 56; Joint Stip. ¶ 10. The examples are all murine, monospecific antibodies of the IgG and IgM isotypes, as well as a number of scFv fragments from some of those antibodies, and one Fab fragment. *See* Marasco Dep. Tr. at 102:12–126:04.

Baxalta’s efforts to produce antibodies that bind to Factor IX/IXa and increase the procoagulant activity of Factor IXa continued until 2003. *See* Cole Decl. vol. 1, Ex. 6, Baxalta’s Suppl. Resp. to Interrog. 11, at 47. The parties agree that Baxalta has never commercialized an antibody for the treatment of hemophilia A in inhibitor patients consistent with the stated purpose of the ’590 patent. *See* Scheiflinger Dep. Tr. at 48:25–49:08; Sheehan Rpt. ¶ 166; Garcia Rpt. ¶ 221; *see also* ’590 patent, col. 2, ll. 25–28 (“[I]t is an object of the [] invention to provide a preparation for the treatment of blood coagulation disorders which has particular advantages for factor VIII inhibitor patients[,] . . . through the use of antibodies . . . against factor IX/IXa.”).

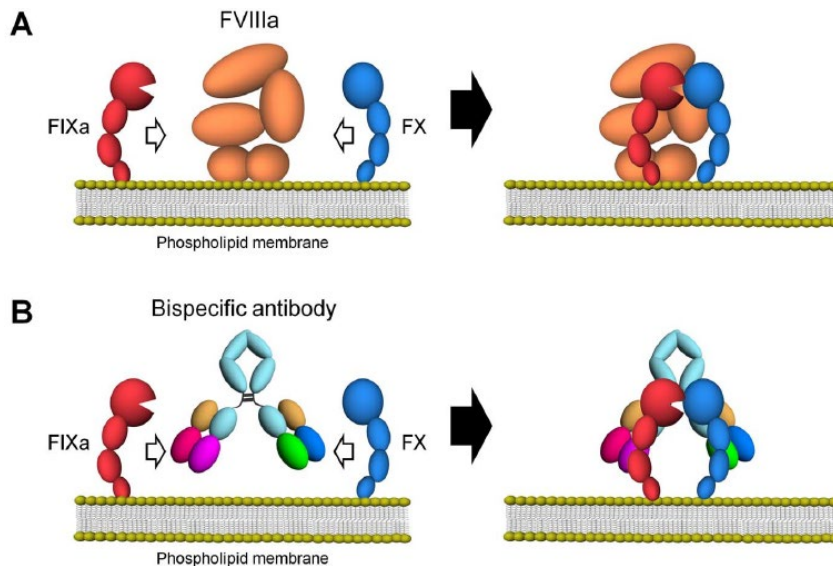
### C. Chugai’s Invention of Emicizumab

Around the same time that Baxalta’s scientists were experimenting with antibodies capable of binding to Factor IX and increasing the procoagulant activity of Factor IXa, scientists in Japan at Chugai were also working to develop antibody-based treatments for hemophilia A. By at least October 27, 2000, Chugai had the idea of using a humanized bispecific antibody that would bind Factor IXa with one arm and Factor X with the other, holding Factor IXa and Factor X in a position

---

<sup>4</sup> The parties dispute whether the patent discloses 11 examples, but for the purposes of its motion for summary judgment, Genentech accepts that the specification discloses 11 working examples. *See* Joint Stip. of Fact, ECF No. 437 (Joint Stip.) ¶ 14.

by which Factor IXa would activate Factor X, similar to how Factor VIIIa functions (depicted below). See Strohl Rpt. ¶ 115; Cole Decl. vol. 1, Ex. 7 at GNE-01138116 (dated Oct. 27, 2000); *id.*, Ex. 8, Kitazawa Dep. Tr. at 185:01–186:10, 215:20–25; *id.*, Ex. 9 at GNE-01137931; *id.*, Ex. 10 at GNE-01137957.



Sampei et al., *Identification and Multidimensional Optimization of an Asymmetric Bispecific IgG Antibody Mimicking the Function of Factor VIII Cofactor Activity*, PLoS ONE 8(2):1–13 (2013) (Sampei Article), at 2, Fig. 1 (showing (A) Factor VIIIa forming a complex with Factor IXa and supporting the interaction between Factor IXa and Factor X through its binding ability to both factors on the phospholipid membrane, and (B) A bispecific antibody (like emicizumab) binding to Factor IXa and Factor X, promoting the interaction between Factor IXa and Factor X and therefore exerting Factor VIII mimetic activity on the phospholipid membrane.).

Genentech's expert, Dr. William R. Strohl, details the lengthy trial-and-error process through which Chugai's scientists generated tens of thousands of combinations of Factor IX and Factor X antibodies, combining them into bispecific antibodies and testing them in assays. Strohl

Rpt. ¶¶ 202–224.<sup>5</sup> Once the scientists had discovered a candidate worthy of testing in animals and then in humans, they engaged in antibody engineering to refine and optimize the candidate antibody. *Id.* ¶¶ 208–210; Strohl Decl., Ex. 2, ECF No. 413-1, Responsive Report, ¶¶ 61–127 (Strohl Resp.). In total, “it took 10 or more full-time Chugai researchers almost 10 years to construct a therapeutically useful bispecific antibody that binds Factor IX/IXa with one arm and Factor X with the other.” Strohl Rpt. ¶ 224.

The resulting antibody, emicizumab, is a humanized bispecific antibody that mimics the function of Factor VIIIa by binding to Factor IXa with one of its arms and to Factor X with the other. Young Decl. ¶ 28; Strohl Resp. ¶ 29. It is the active ingredient in Hemlibra—the first and only FDA-approved product for hemophilia A patients that can be injected under your skin (subcutaneously). Young Decl. ¶ 28; Malackowski Rpt. at 38. Hemlibra has been shown to increase procoagulant activity to about 10% of normal Factor VIII levels. *See* Decl. of Stephanie A. Smith, ECF No. 420, Ex. 1 at ¶ 49 (citing Uchida et al., *A first-in-human phase I study of ACE910, a novel factor VIII-mimetic bispecific antibody, in healthy subject*, *Blood* 127(13):1633–1641 (2016)); *Id.* at Ex. 2, ¶ 44. This is enough to move a patient from severe hemophilia A (with observed Factor VIII activity less than 1%) to at least a moderate category (with observed factor VIII activity between 1–5%) or even a mild category (with observed factor VIII activity between 5–40%). *See* Smith Decl., Ex. 2, ¶ 44; Krishnaswamy Rpt. ¶ 117 (showing severity classifications).

---

<sup>5</sup> The work conducted by Chugai’s scientists to discover emicizumab also is documented in literature. *See* Sampei Article; *see also* Sampei et al. (2013) Discussion, *available at* <https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0057479> (explaining that “the lead bispecific antibody was identified from approximately 40,000 different bispecific antibodies” and “[b]ispecific antibodies meeting the criteria for FVIII cofactor activity were extremely rare (<0.3%)”).

On November 16, 2017, the FDA approved Hemlibra for routine prophylaxis to prevent or reduce the frequency of bleeding episodes in adult and pediatric patients with hemophilia A with Factor-VIII inhibitors. Young Decl. ¶ 28. On October 4, 2018, the FDA approved Hemlibra for non-inhibitor patients. *Id.*

#### V. Enablement Standard

A patent claim is presumed enabled unless proven otherwise by clear and convincing evidence. 35 U.S.C. § 282; *Ormco Corp. v. Align Tech., Inc.*, 498 F.3d 1307, 1318 (Fed. Cir. 2007). The central question for enablement is whether the specification enables the full scope of its claims without undue experimentation. *Plant Genetic Sys., N.V. v. DeKalb Genetics Corp.*, 315 F.3d 1335, 1339 (Fed. Cir. 2003). “Enablement is not precluded where a ‘reasonable’ amount of routine experimentation is required to practice a claimed invention.” *ALZA Corp. v. Andrx Pharm., LLC*, 603 F.3d 935, 940 (Fed. Cir. 2010). To evaluate whether the patent enables a person of ordinary skill in the art to practice the invention without undue experimentation, courts consider a non-exclusive list of items, often referred to as the *Wands* factors: “(1) the quantity of experimentation necessary, (2) the amount of direction or guidance presented, (3) the presence or absence of working examples, (4) the nature of the invention, (5) the state of the prior art, (6) the relative skill of those in the art, (7) the predictability or unpredictability of the art, and (8) the breadth of the claims.” *In re Wands*, 858 F.2d 731, 737 (Fed. Cir. 1988). A court need not consider each of the *Wands* factors, for they “are illustrative, not mandatory.” *Amgen, Inc. v. Chugai Pharm. Co.*, 927 F.2d 1200, 1213 (Fed. Cir. 1991).

## VI. Undisputed Facts Relevant to Enablement

The following are undisputed material facts, based on the patent claims, the specification, the court's claim construction order, Genentech's Motion for Summary Judgment, Baxalta's response, and the parties' stipulation dated December 3, 2021:

1. Claim 1 of the '590 patent encompasses any isolated antibody or antibody fragment thereof that binds Factor IX or Factor IXa and increases the procoagulant activity of Factor IXa by any amount. *See* Claim Construction Order at 29.
2. The number of candidate antibodies or antibody fragments within the scope of claim 1 is high, "encompassing millions of different structural formats, binding epitopes, binding affinities, and mechanisms of action." Garcia Rpt. ¶ 215. The specification discloses working examples of only eleven antibodies that satisfy claim 1. *See* Joint Stip. ¶ 14.
3. The structural breadth of claim 1 is illustrated by its dependent claims. The dependent claims show that claim 1 is not specific to any particular isotype of the antibody or antibody fragment and includes antibodies that have been genetically engineered into different structural formats.
  - a. Dependent claims 3 and 20, define *Markush* groups<sup>6</sup> and include antibodies of the IgA, IgE, IgG and IgM isotypes that are within the scope of claim 1. Claim 1 also includes the fifth principal isotype, IgD, because claim 1 is not limited by isotype. *See* Def.'s Mot. at 18; Marasco Dep. Tr. at 103:12–104:9. The patent does not disclose working examples of three of these isotypes: IgD, IgA,

---

<sup>6</sup> "A Markush group lists specified alternatives in a patent claim, typically in the form: a member selected from the group consisting of A, B, and C." *Gillette Co. v. Energizer Holdings, Inc.*, 405 F.3d 1367, 1372 (Fed. Cir. 2005) (citing to *Manual of Patent Examining Procedure* § 803.2 (2004)).



and IgE. *See* Marasco Dep. Tr. at 102:08–22; 105:06–09; 105:20–106:12.

The two isotypes represented by working examples are those that are most commonly present in high proportion in the blood at the early stages of an immune response. Marasco Rpt. ¶ 71; Strohl Rpt. ¶ 37. Using the teachings of the '590 patent, it would be rare to discover antibodies of the IgA and IgE isotypes, as the vast majority of antibodies, 75 percent, exist as IgG and IgM isotypes. It would be rarer still to discover IgD antibodies using the teachings of the '590 patent, since they do not circulate in the bloodstream but are instead bound to the exterior membranes of immune-system cells. *See* Marasco Dep. Tr. at 104:13–105:5.

- b. Dependent claims 4 and 19 define *Markush* groups that include various types of antibodies, namely monoclonal antibodies, chimeric antibodies, humanized antibodies, single chain antibodies (such as scFvs), bispecific antibodies, and diabodies, as well as dimers, oligomers, and multimers thereof. The '590 patent does not disclose working examples of seven of the nine structural formats in the *Markush* group of claims 4 and 19: chimeric antibodies, humanized antibodies, bispecific antibodies, diabodies, and dimers, oligomers and multimers thereof. *See* Marasco Dep. Tr. at 124:24–125:12, 126:02–04; Scheiflinger Dep. Tr. at 66:16–17.

4. Claim 1 also is functionally broad. An antibody that increases the amount of procoagulant activity by the same amount as Factor VIII does (at least 40%) is within the scope of the asserted claims. *See* Marasco Dep. Tr. at 236:12–18; Cole Decl. vol. 2, ECF No. 410-1, Ex. 20, Krishnaswamy Dep. Tr. at 213:17–214:11. The highest estimated amount by which

any antibody disclosed in the '590 patent increased the procoagulant activity of Factor IXa was by 3.75% (antibody 198/A1)—far less than 40%.<sup>7</sup> See Krishnaswamy Rpt. ¶¶ 122–123. The 3.75% would only be capable of moving a patient with hemophilia A classified as severe to a moderate classification, pursuant to the below chart upon which both parties' experts rely.

Factor VIII Activity	Classification
< 0.01 IU/mL (< 1% of normal)	Severe
0.01 – 0.05 IU/mL (1%–5% of normal)	Moderate
> 0.05 – < 0.40 IU/mL (>5% – <40% of normal)	Mild

See Krishnaswamy Rpt. ¶¶ 117; Sheehan Rpt. ¶ 99. The specification does not disclose an antibody or antibody fragment that is therapeutically useful for moving someone suffering from a severe case of hemophilia A to a mild case. Patients with severe conditions represent about 60 percent of hemophilia A cases. Malackowski Rpt. at 22.

5. Claim 1 also includes antibodies or antibody fragments that are capable of increasing the procoagulant activity of Factor IXa in the presence of inhibitors (as specified in claim 2). The specification states that the objective of the patent is “to provide a preparation for the treatment of blood coagulation disorders which has particular advantages for factor VIII inhibitor patients.” '590 patent, col. 2, ll. 25–28; *id.* at col. 2, ll. 29–45.
6. The '590 patent discloses only one working example of an antibody shown to increase the procoagulant activity of Factor IXa in the presence of Factor VIII inhibitors as required by claim 2 (antibody 193/AD3). See Marasco Dep. Tr. at 122:06–11; Marasco Rpt. ¶ 73. The highest amount that the 193/AD3 antibody increased the procoagulant activity of Factor

---

<sup>7</sup> The 3.75% figure does not appear in the specification; a person of ordinary skill in the art reading the specification could derive it from the information disclosed in Figure 25. See Krishnaswamy Rpt. ¶ 123.

IXa by was only 0.3–0.4% equivalent Factor VIII activity—a marginal amount. *See* Sheehan Rpt. ¶ 99; Krishnaswamy Rpt. ¶ 118.

7. In order to treat hemophilia A without a HAMA response, it would be necessary to utilize a humanized, or at least chimeric, antibody. *See* Strohl Rpt. ¶¶ 50–56.<sup>8</sup> There are no working examples of humanized or chimeric antibodies disclosed in the patent specification. *See* Marasco Dep. Tr. at 124:24–125:12, 126:02–04; Scheifflinger Dep. Tr. at 66:16–17.
8. The inventors of the '590 patent performed their experimentation for a period of three to four years and never brought to market an antibody within the scope of claim 1 for the treatment of hemophilia A. *See* Scheifflinger Dep. Tr. at 48:25–49:08; Kerschbaumer Dep. Tr. at 14:325–15:328; Sheehan Rpt. ¶ 166.
9. Under the teachings of the '590 patent, arriving at an antibody that binds to Factor IX or IXa and increases the procoagulant activity of Factor IX is a multi-step process, involving experimentation at every critical step. *See infra* ¶¶ 10–27.
10. The level of skill in the art for the '590 patent is high,<sup>9</sup> Pl.'s Opp'n at 28–29 (citing Marasco Rpt. ¶¶ 264–265), and a person of ordinary skill in the art would be familiar with the

---

<sup>8</sup> *See also* Morrison, et al., *Chimeric human antibody molecules: mouse antigen-binding domains with human constant region domains*, Proc. Natl. Acad. Sci. USA 81, 6851–55 (1984), cited in Marasco Rpt. ¶ 286 n.254.

<sup>9</sup> There is no material difference between the parties' descriptions of the level of skill in the art. *See* Garcia Decl. ¶¶ 52, 53. The court adopts Baxalta's definition for the purposes of this motion. That is, a person of ordinary skill in this art is one who:

would have had an advanced degree and relevant work experience, either an M.D. and several years' experience practicing in the area of hematology or a Ph.D. in a chemical science- or biological science-related discipline. This person would have a working knowledge of experimental methodologies for detecting the activity of factors in the clotting cascade, measuring blood clotting

technology and techniques discussed in the patent for producing and testing antibodies generally, Marasco Rpt. ¶¶ 264–265. But it would not be possible for a person skilled in the art to predict which antibodies would satisfy the claim limitations without trial-and-error testing. *See* Scheiflinger Dep. Tr. at 92:15–93:3; Marasco Dep. Tr. at 205:04–19; Krishnaswamy Dep. Tr. at 168:09–168:19.

11. There is no guidance or direction in the specification of the '590 patent as to how to identify antibodies that satisfy the claim limitations except by using trial and error. *See* Marasco Dep. Tr. at 205:04–19; Krishnaswamy Dep. Tr. at 168:09–168:19; Scheiflinger Dep. Tr. at 92:15–93:3.
12. “The only way to know [what antibodies bind as well as function as needed] is to make antibodies and test them.” Krishnaswamy Dep. Tr. at 168:20–169:02; Marasco Dep. Tr. at 218:23–219:04.<sup>10</sup> The '590 patent does not describe what structural or other features of the disclosed antibodies cause them to bind to Factor IX/IXa or to increase the procoagulant activity of Factor IXa. *See* Garcia Rpt. ¶ 130; Scheiflinger Dep. Tr. at 91:23–92:3; 97:23–98:02.

---

capabilities by a variety of means, or would have general familiarity with basic concepts in immunology, including basic knowledge of methods for making antibodies and using them as therapeutics. This hypothetical person would be teamed with or have access to other highly skilled individuals with advanced degrees (*e.g.*, Ph.Ds.) in other biological disciplines such as immunology or molecular biology who had several years' experience with methods to produce antibodies that bind to antigens of interest.

*Id.* ¶ 53; Marasco Rpt. ¶ 264.

<sup>10</sup> “Q. . . . [T]he only way that the patent teaches a person of ordinary skill how to tell whether a given antibody to Factor IX or Factor IXa, in fact, increases the procoagulant activity of Factor IXa is to test that antibody in an assay, correct? A. That's what the patent teaches.”

13. At step one of the multi-step process for producing antibodies that bind to Factor IX or IXa and increase the procoagulant activity of Factor IXa, the specification discloses how to produce antibodies using one of several methods known in the prior art “(e.g., by conventional hybridoma techniques, or by means of phage display gene libraries, immunoglobulin chain shuffling or humanizing).” ’590 patent, col. 7, l. 66–col. 9, l. 10.
14. The antibodies disclosed in the ’590 patent were generated using the “hybridoma” technique, as shown in Example 1. *See* Joint Stip. ¶¶ 1, 6; ’590 patent, col. 9, l. 66–col. 10, l. 37.
15. In the hybridoma process, mice in groups of one to three are injected with Factor IX or IXa over a period of days. Joint Stip. ¶¶ 3, 7; ’590 patent, col. 9, l. 66–col. 10, l. 8.
16. The mouse’s immune system responds to the Factor IX injections by producing B-cells in its spleen that secrete antibodies against the human antigen. Joint Stip. ¶ 3. Each B-cell produces only a single antibody. *Id.* It is not possible to predict whether a mouse used to make hybridomas will produce antibodies that satisfy the claim requirements. *See* Scheiflinger Dep. Tr. at 92:15–93:03.
17. Each mouse is then euthanized, and its spleen cells removed. *See* ’590 patent, col. 10, l. 8–9; Joint Stip. ¶ 4. In order to enable murine antibodies to survive and to replicate themselves sufficiently for further experimentation, the spleen cells are fused with myeloma (cancer) cells through a process known in the prior art. ’590 patent, col. 10, l. 9–11; Joint Stip. ¶ 5. The inventors performed and disclosed in the ’590 patent at least four such “fusion” experiments, which they labeled #193, 195, 196, and 198. Joint Stip. ¶ 8 (citing ’590 patent, col. 10, ll. 11–13).

18. In each fusion experiment, after the B-cells were fused with the myeloma cells, the resulting hybrid cells, or “hybridomas,” were isolated and screened using techniques known in the prior art to determine whether they produce antibodies that bind to the antigen of interest (in this case, Factor IX or IXa). *See* Joint Stip. ¶¶ 5, 9; Garcia Rpt. at 30, Fig. 8; ’590 patent, col. 10, ll. 14–31.
19. Not all of the antibodies produced at step one will bind to Factor IX/IXa. Oral Arg. Hr’g Tr., ECF No. 431 (Hr’g Tr.), at 28:16–18; Scheifflinger Dep. Tr. at 92:15–93:3. Around “60% of the hybridoma cell lines screened expressed an FIX-binding antibody.” Garcia Decl., Ex. 2, Reply Rpt. ¶ 21 n. 15 (citing Scheifflinger, F. et al., *Enhancement of the enzymatic activity of activated coagulation factor IX by anti-factor IX antibodies*, J. Thromb Haemost 6(2):315–322 (2008)).
20. Once the antibodies are filtered to only those that bind to factor IX/IXa, they must undergo additional screening to determine which among them demonstrate the ability to increase the procoagulant activity of Factor IXa. Garcia Rpt. ¶ 213; Marasco Dep. Tr. at 218:23–219:04.
21. In terms of the method used to measure procoagulant activity, the patent provides that “all the methods used for determining Factor VIII activity may be used.” ’590 patent, col. 9, ll. 22–25; *see also* Claim Construction Order at 29 (construing “increases the procoagulant activity of Factor IXa” to mean “[t]he ability of Factor IXa to activate Factor X to Factor Xa by any amount as determined *by any assay used* to measure Factor VIII-like activity” (emphasis added)).
22. The specification of the ’590 patent recommends the use of a modified version of the commercially available chromogenic test-kit called COATEST VIII:C/4® (COATEST)

for the hybridoma screening step. *See* '590 patent, col. 10, l. 40–col. 12, l. 56. The modified protocols disclosed in the examples of the '590 Patent are significantly different from the recommended protocol for the commercially available COATEST test. Sheehan Rpt. ¶ 150; Krishnaswamy Rpt. ¶¶ 141, 157–161. The modifications that the inventors made to the test were designed to make the test more sensitive. *See* Marasco Dep. Tr. at 219:5–15. They also made the test more complex and time consuming. *See* '590 patent, col. 10, ll. 48–67, col. 15, ll. 44–45; Krishnaswamy Rpt. ¶ 158; Sheehan Rpt. ¶ 150. Whereas the standard COATEST assay takes minutes, the modification disclosed in the patent takes several hours. *See* Sheehan Rpt. ¶ 150, Krishnaswamy Rpt. ¶ 158; '590 patent, col. 10, ll. 48–67, col. 15, ll. 44–45.

23. The inventors of the '590 patent did not use any of the other commonly used methods to screen hybridoma cells for procoagulant activity and did not determine or describe in the specification what modifications would be necessary for those other tests to function. '590 patent, col.9, ll. 22–25; Garcia Rpt. ¶ 220.
24. In an article published by the inventors after the '590 patent was filed, they disclosed that the vast majority of antibodies produced and screened in experiments leading up to the '590 patent (98.4% of them) did not increase the procoagulant activity of Factor IXa by any amount. *See* Cole Decl. vol. 2, Ex. 24, Scheifflinger, et al., *Enhancement of the Enzymatic Activity of Activated Coagulation Factor IX by Anti-Factor IX Antibodies* (Scheifflinger Article), at 320; Marasco Dep. Tr. at 202:18–21 (agreeing that the inventors reported only 1.6% of the antibodies had procoagulant activity); Marasco Rpt. ¶ 262 (“the number [of antibodies] that would activate Factor IXa such that there is an increase in procoagulant activity, is a very, very minor sub-fraction.”).

25. Once the inventors discovered antibodies that increased the procoagulant activity of Factor IXa by screening the hybridoma cells using the modified COATEST assay, they then tested one of those antibodies (193/AD3) in aPTT assays to measure clotting time, including in the presence of Factor VIII inhibitors. '590 patent, col. 16, l. 44–col. 17, l. 67; Figures 9, 10A, 10B.
26. There are no examples in the patent of seven of the nine structural formats falling under claim 4 (a chimeric antibody, a humanized antibody, a bispecific antibody, a diabody, or di-, oligo- or multimers thereof). *See* Marasco Dep. Tr. at 102:12–126:04. In order to arrive at those antibody formats from the antibodies produced through the aforementioned steps, it would be necessary for one skilled in the art to genetically modify them. *See supra* § IV.B.1. This was never done, and the patent does not provide specific guidance on how such modification would take place. Although a person skilled in the art would be familiar with the procedures for modifying antibodies using techniques known in the prior art, additional confirmatory testing would have been necessary following modification to ensure that the binding and activating functions of the antibody remained in place. *See* Marasco Rpt. ¶¶ 235, 275; *see also* Garcia Rpt. ¶ 212; Marasco Dep. Tr. at 127:24–128:25.
27. For example, the process for humanizing antibodies was well-known in the art prior to 1999, *see* Marasco Rpt. ¶ 289 n. 258 (citing Strohl Dep. Tr., ECF No. 424-11, at 17:24–18:9), but the process was “not as efficient [] as sometimes presented,” Marasco Dep. Tr. at 130:18–19. The process involves selecting “human framework regions . . . from heavy chain and light chain sequences of over 1,000 human sequences each,” *id.* at 130:20–22, and “the resulting antibody, despite having the same variable region as the murine antibody, frequently does not have the same effectiveness as the original murine antibody,”



*id.* at 130:23–131:01. Given this uncertainty, additional screening would be required to confirm whether there had been any degradation in the binding or activating functions of the antibody. *See id.* at 132:10–12; Marasco Rpt. ¶ 275.

28. The accused product, emicizumab, is a bispecific humanized antibody that mimics Factor VIIIa by binding Factor IXa with one arm and Factor X with the other arm. *See* Sampei Article at 1. It took the scientists at Chugai almost 10 years to develop emicizumab. Strohl Rpt. ¶ 224. They underwent a multi-phased, trial-and-error process that involved screening tens of thousands of antibodies and engineering the resulting antibodies for optimization before finding one that was suitable for clinical use. *Id.* ¶¶ 212–224; *see also* Sampei Article at 1–13. “The lead bispecific antibody was identified from approximately 40,000 different bispecific antibodies” and “[b]ispecific antibodies meeting the criteria for FVIII[-like] activity were extremely rare (<0.3%).” Sampei et al. (2013) Discussion, *available at* <https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0057479>.

## VII. Application of the *Wands* Factors

Applying the *Wands* factors here, the court finds as a matter of law that undue experimentation would be needed to practice the full scope of the claimed invention. First, with respect to “the quantity of experimentation necessary” (factor 1), Baxalta does not dispute that practicing the teachings of the ’590 patent involves a large amount of experimentation. The potential candidates number in the millions. *See supra* § VI ¶ 2. As discussed, the patent teaches a multi-step process, with screening at every critical step to determine antibodies within the scope of the claims. *See id.* ¶¶ 13–27. Turning to factor 2, there is a limited “amount of guidance presented in the patent.” *See id.* ¶ 11. There is no guidance or direction as to how to identify antibodies that satisfy the claims’ limitations other than by utilizing trial and error. *See id.* This

lack of guidance is compounded by a limited number of “working examples” (factor 3). While the specification of the ’590 patent discloses eleven working examples, they are all monospecific murine antibodies or fragments thereof that bind to Factor IX or IXa and increase the procoagulant activity of Factor IXa by a small amount. *See id.* ¶¶ 1–4. It does not disclose working examples of antibodies of the IgE, IgA, or IGD isotypes; of humanized, chimeric, or bispecific antibodies; of diabodies; or of dimers, oligomers or multimers thereof. *See id.* ¶ 3. There also is no working example of an antibody that increases the procoagulant activity of Factor IXa by an amount capable of moving a patient with a severe case of hemophilia A to a mild case. *See id.* ¶ 4. There is not a single example of an antibody that produces procoagulant activity in the presence of Factor VIII inhibitors by more than a marginal amount. *See id.* ¶¶ 4–6.

Courts often consider factors 4 and 7 (the “nature of the invention” and the “predictability or unpredictability” of the art) together. *See, e.g., Alza Corp. v. Andrx Pharms., LLC*, 607 F. Supp. 2d 614, 655–56 (D. Del. 2009). This area of art is inherently unpredictable. The field of antibodies is itself unpredictable. *See Centocor Ortho Biotech, Inc. v. Abbott Labs.*, 636 F.3d 1341, 1352 (Fed. Cir. 2011) (analogizing finding an appropriate antibody for a particular antigen to searching for a key “on a ring with *a million* keys on it” (internal citations and quotation marks omitted)). That unpredictability is compounded here by the lack of guidance as to how to produce antibodies satisfying the full scope of the claims other than by trial-and-error. *See Fisher*, 427 F.2d at 839 (“In cases involving unpredictable factors, such as most chemical reactions and physiological activity, the scope of enablement obviously varies inversely with the degree of unpredictability of the factors involved.”).

Turning to factor 6—the “relative skill of those in the art”—the level of skill would be high, with a POSITA holding “an advanced degree and relevant work experience, either an M.D.

and several years’ experience practicing in the area of hematology or a Ph.D. in a chemical science- or biological science-related discipline.” *Supra* note 10. Given the “state of the prior art” (factor 5), a POSITA would be familiar with the techniques for producing antibodies using hybridoma or phage display technology and in using the standard chromogenic or aPTT assays used in the trial and error process, *see supra* § VI ¶ 10, but could not predict in advance which antibodies would satisfy the claim limitations, *see id.* ¶ 11.

Finally, turning to factor 8, the undisputed facts show that a reasonable factfinder could only find that the “breadth of the claims” is great. By Baxalta’s experts’ own admissions, claim 1 covers all antibodies and fragments of any format, isotype or subtype, that bind with any affinity to factor IX/IXa, that achieve procoagulant effect through any mechanism of action, and that demonstrate procoagulant activity ranging from minuscule to therapeutically useful amounts, with or without the presence of Factor VIII inhibitors. *See id.* ¶¶ 1–5.

Decisions of the Federal Circuit applying the *Wands* factors make clear that the claims asserted here are not enabled.

#### VIII. Enablement of the Full Scope of the Asserted Claims of the ’590 Patent

##### A. Make-and-Screen Nature of Invention

*First*, where, as here, there are a large number of potential candidates, few working examples disclosed in the patent, and no guidance in the specification as to how to practice the full scope of the invention except to use trial and error to narrow down the potential candidates to those satisfying the claims’ functional limitations—the asserted claims are not enabled. *See Idenix Pharms. LLC v. Gilead Scis. Inc.*, 941 F.3d 1149, 1155–56 (Fed. Cir. 2019) (finding nonenablement where the claims included the broad functional limitation of having efficacy against hepatitis C virus, which required screening a large number of candidates to identify

compounds that satisfied the limitation); *Enzo Life Scis., Inc. v. Roche Molecular Sys., Inc.*, 928 F.3d 1340, 1346–47 (Fed. Cir. 2019) (finding nonenablement where the claims required both a particular structure and functionality but the specification failed to teach one of skill in the art whether the many embodiments of the broad claims would exhibit that required functionality); *Wyeth & Cordis Corp. v. Abbott Labs.*, 720 F.3d 1380, 1385–86 (Fed. Cir. 2013) (finding, due to the large number of possible candidates within the scope of the claims and the specification’s corresponding lack of structural guidance, it would have required undue experimentation to synthesize and screen each candidate to determine which compounds in the claimed class exhibited the claimed functionality); *see also McRO*, 959 F.3d at 1100 n.2 (“In cases involving claims that state certain structural requirements and also require performance of some function (*e.g.*, efficacy for a certain purpose), we have explained that undue experimentation can include undue experimentation in identifying, from among the many concretely identified compounds that meet the structural requirements, the compounds that satisfy the functional requirement.”).

In this respect, the facts of this case are strikingly similar to the facts of *Amgen Inc. v. Sanofi, Aventisub LLC*, 987 F.3d 1080 (Fed. Cir. 2021). There, as here, the claims were directed to a genus that was claimed broadly in terms of functionality. The two patents at issue there were directed to monoclonal antibodies for use in treatment of elevated low-density lipoprotein (“LDL”) cholesterol—a leading cause of heart disease. *Amgen*, 987 F.3d at 1082–83. The body removes LDL cholesterol from the blood stream using LDL receptors. *Id.* at 1082. But “PCSK9,” a naturally occurring protein, can bind to LDL receptors and cause the receptors to be destroyed, an undesirable result. *Id.* at 1082–83. The antibodies disclosed in Amgen’s patents were claimed to prevent the degradation of LDL receptors by binding a specific region of PCSK9. *Id.* at 1083. By binding that specific region, the antibodies block PCSK9 from binding LDL receptors and causing

them to be destroyed. *Id.* The asserted claims thus imposed a functional limitation that the antibodies bind to a specific target.<sup>11</sup> The specification disclosed working examples of 26 antibodies that satisfied the claim limitations. *Id.*

In assessing enablement, the court first explained there are “high hurdles in fulfilling the enablement requirement for claims with broad functional language.” *Id.* at 1087. Applying that standard, the court agreed with the district court’s finding that the specification did not enable preparation of the full scope of the asserted claims—that the antibodies bind a specific target. *Amgen*, 987 F.3d at 1087. Key to that determination was the court’s finding that the “claims [we]re far broader in functional diversity than the disclosed examples,” and “the only ways for a person of ordinary skill to discover undisclosed claimed embodiments would be through either ‘trial and error, by making changes to the disclosed antibodies and then screening those antibodies for the desired binding and blocking properties,’ or else ‘by discovering the antibodies *de novo*’ according to a randomization-and-screening ‘roadmap.’” *Id.* at 1088 (quoting *Amgen Inc. v. Sanofi*, 2019 WL 4058927, at \*11 (D. Del. 2019)); *see also Idenix*, 941 F.3d at 1161 (“A specification that requires a [POSITA] to ‘engage in an iterative, trial-and-error process to practice the claimed invention’ does not provide an enabling disclosure.” (quoting *ALZA*, 603 F.3d at 941)).

*Amgen*’s reasoning applies with equal force here, where the asserted claims also set forth not one but two functional requirements: that the antibodies bind to a target (Factor IX or IXa) and alter that target’s activity (increasing the procoagulant activity of Factor IXa). Marasco Rpt. ¶ 63 (agreeing with Genentech’s expert, Dr. K. Christopher Garcia, that the claims include the two functional limitations). Even if the first functional requirement (binding) were enabled, the second

---

<sup>11</sup> Although the asserted claims also included a functional limitation of “blocking the PCSK9/LDLR interaction,” *Amgen*, 987 F.3d at 1083, the court found that “[t]he binding limitation [alone] [wa]s [] enough [] to require undue experimentation,” *id.* at 1087.

is not. The record shows that if a person of ordinary skill in the art (POSITA) started with the genus of antibodies and antibody fragments that bind to Factor IX or IXa, only a very tiny percentage of those will meet the claims' functional limitation that the antibody or antibody fragment increase the procoagulant activity of Factor IXa,<sup>12</sup> and the only way to find that small number within the larger whole (of potentially millions of combinations satisfying the structural requirements), given the inherent unpredictability of the art and the lack of guidance in the specification, is by screening tens of thousands (if not more) antibodies or antibody fragments for procoagulant activity. *See supra* § VI ¶¶ 2, 10–24. It is a search for a needle in a haystack.

While Baxalta does not dispute the breadth of the claims, it asserts that only a “very, very minor sub-fraction” of antibodies will satisfy the claim limitations. Pl.’s Opp’n at 12 (quoting Garcia Rpt. ¶ 189). That is true, but this only exemplifies how substantial experimentation is necessary to sift through the broad genus of possible candidates to find the narrow species that satisfy the claim limitation.

The Federal Circuit considered an almost identical theory in *Idenix*, where the court found the claims were invalid for lack of enablement. 941 F.3d at 1161–63. The patent in *Idenix* claimed a method of treatment for the hepatitis C virus (HCV) by using a particular pharmaceutical drug.

---

<sup>12</sup> Although the results of the '590 patent inventors' experimentation (showing only 1.6% of antibodies screened bound to Factor IX/IXa and increased the procoagulant activity of Factor IXa) are not disclosed in the patent, the court may consider extrinsic evidence of those results to support its finding of non-enablement. *See Pharm. Res., Inc. v. Roxane Labs., Inc.*, 253 F. App'x 26, 31 n.3 (Fed. Cir. 2007) (“Although extrinsic evidence cannot be used to supplement a non-enabling specification, such evidence can shed light on whether the specification is itself enabling.”); *see also Atlas Powder Co. v. E.I. du Pont De Nemours & Co.*, 750 F.2d 1569, 1577 (Fed. Cir. 1984) (considering results of experiments performed by patentee prior to filing the patent); *Enzo Biochem, Inc. v. Calgene, Inc.*, 188 F.3d 1362, 1373 (Fed. Cir. 1999) (determining evidence of the patentee's own experimental failures was appropriate to consider).

The patent claimed the drug in a generic manner that included a large number of related compounds. The only independent claim of the patent recited:

1. A method for the treatment of a hepatitis C virus infection, comprising administering an effective amount of a purine or pyrimidine  $\beta$ -D-2'-methyl-ribofuranosyl nucleoside or a phosphate thereof, or a pharmaceutically acceptable salt or ester thereof.

*Id.* at 1155 (quoting U.S. Patent No. 7,608,597).

Of the “billions of potential 2'-methyl-up nucleosides,” the specification identified only a small subset (four) compounds as being effective. *Id.* at 1156, 1161. The court thus found that the broad functional limitation of having efficacy against hepatitis C virus (HCV) meant that there were a very large number of potential nucleoside candidates and only a few examples that satisfied the claim limitation. *Id.* at 1155–56, 1162. Idenix argued that the claims were not broad because, “[w]hen required to take all of the claim limitations into account, Gilead’s witnesses described the claims as embracing only a ‘small’ number of compounds.” *Id.* at 1162.

The court rejected that analysis as “backwards,” explaining that “to get from a large number of candidate compounds to a relatively speaking small number of effective compounds . . . leaves a [POSITA] searching for a needle in a haystack to determine which of the ‘large number’ of . . . [candidate compounds] falls into the ‘small’ group of candidates that effectively treats HCV.” *Id.* “The size disparity between those two groups,” the court reasoned, “requires significant experimentation, which weighs against enablement, not for it.” *Id.* The same reasoning applies here where the only way to find the very small number of antibodies that meet the claims’ broad functional requirements, among millions of possible combinations, is to experiment. That there are few needles in the haystack makes the search harder, not easier.

Baxalta argues that that the experimentation required to practice the full scope of the invention is not undue here because “a POSITA need only engage in *routine* experimentation,” as

shown in Examples 1 and 2 of the specification. Pl.’s Opp’n at 22 (emphasis added) (quoting Marasco Rpt. ¶ 214).<sup>13</sup> The same was true in *Amgen*, where expert testimony showed that “a person of skill in the art c[ould] make all antibodies within the scope of the claims by following a roadmap using anchor antibodies and well-known screening techniques as described in the specification or by making conservative amino acid substitutions in the twenty-six examples.” *Amgen*, 987 F.3d at 1085. The court nevertheless found the experimentation was undue. *Id.* at 1088.

The Federal Circuit also considered and rejected a similar argument in *Wyeth*. Like Baxalta, Wyeth argued that practicing the full scope of the claims would not require undue experimentation because it “would have required only *routine* experimentation.” 720 F.3d at 1384 (emphasis added). The Federal Circuit disagreed, explaining that even taking all of Wyeth’s contentions—including that “one of ordinary skill could routinely use the assays disclosed in the specification to determine” which compounds fall within the scope of the claims, the claims were not enabled because “there [we]re still at least tens of thousands of candidates” to screen. *Id.* at 1385.

The same reasoning dooms Baxalta’s routine experimentation arguments here. There is no guidance or direction in the specification of the ’590 patent as to how to distinguish antibodies that bind to Factor IXa and increase the procoagulant activity of Factor IXa from those that do not. *Supra* ¶ 10. And “there is no genuine dispute that it would be necessary to first synthesize and then screen *each* candidate compound using the assays disclosed in the specification to determine

---

<sup>13</sup> Baxalta also points to its expert’s statement that “[t]he specification instructs a POSITA to use well-known, inexpensive, and efficient means of producing an antibody or fragment thereof that binds to Factor IX or Factor IXa and identifying those that increase the procoagulant activity of Factor IXa,’ [and] it would not require ‘substantial time and effort’ to make and use the claimed invention.” Pl.’s Opp’n at 22 (quoting Marasco Rpt. ¶ 241).



whether it has” procoagulant effect, and until you screen the antibodies, “you can’t tell whether they work or not.” *Wyeth*, 720 F.3d at 1385.

Finally, while acknowledging that “a POSITA would need to screen for procoagulant activity,” Pl.’s Opp’n at 22 (citing Marasco Dep. Tr. at 204:01–205:19), Baxalta argues that the requisite experimentation is nevertheless not undue because “POSITAs would ‘feel confident that they could [utilize the teaching of the patent to produce] antibodies [] that have procoagulant return,’” *id.* (quoting Marasco Dep. Tr. at 209:02–09). Baxalta quotes one of its experts’ testimony that there is a “‘profound[]’ difference between (i) making antibodies that bind to Factor IX/IXa and screening them for procoagulant activity, and (ii) starting from scratch or through trial and error.” *Id.* at 22 (quoting Marasco Dep. Tr. at 206:07–13); *see also* Marasco Dep. Tr. at 206:14–19 (“A. . . . It’s no longer an unknown of maybe I’ll find them.”). But this was also true in the *Idenix*, *Enzo*, *Wyeth* and *Amgen* cases, where the inventors had identified a small number of compounds within the scope of the claims and the court found the claims were not enabled given the breadth of the claims and the lack of sufficient guidance in the specifications.<sup>14</sup> There is nothing in the specification teaching how to identify any antibodies complying with the claim limitations other than by repeating the same process the inventors used to identify the eleven examples disclosed in the specification.

---

<sup>14</sup> Baxalta also attempts to distinguish *Amgen* on the basis that the claims there specified the amino acids (or residues) on PCSK9 to which the antibodies must bind. Pl.’s Opp’n at 32 (citing *Amgen*, 987 F.3d at 1087 n.1 (“For example, there are three claimed residues to which not one disclosed example binds.”)). Baxalta maintains “[t]here is no similar unpredictability or lack of enablement in the” asserted claims in this case because they “merely require binding to Factor IX/IXa and do not require binding to specific residues.” Pl.’s Opp’n at 32. The court disagrees. Here, similar to *Amgen*, there are various categories of antibodies that are identified in the claims that are not represented by working examples.

## B. Broad Functional Scope

*Second*, the patent claims in this case, even more so than those in *Amgen* (which focused only on the binding requirement), cover a wide range of functionality in terms of procoagulant activity and that range is not represented by working examples. The Federal Circuit’s cases make clear that where, as here, “a range is claimed, there must be reasonable enablement of the scope of the range.” *See Amgen*, 987 F.3d at 1085 (quoting *McRO, Inc. v. Bandai Namco Games Am. Inc.*, 959 F.3d 1091, 1100 (Fed. Cir. 2020)); *see also MagSil Corp. v. Hitachi Global Storage Techs., Inc.*, 687 F.3d 1377, 1384 (Fed. Cir. 2012) (claims not enabled where the patentee argued for a broad scope despite meager results achieved by the inventors). By Baxalta’s own admission, the patent covers everything from a barely perceptible amount of procoagulant activity at the bottom end to an amount that would be created by Factor VIII itself (at least 40%) at the upper end. *See Marasco Dep. Tr.* at 236:12–18;<sup>15</sup> *Krishnaswamy Dep. Tr.* at 213:17–214:11;<sup>16</sup> *see also supra* § VI ¶ 4.

The Federal Circuit’s decision in *MagSil* is instructive. There, a patentee asserted infringement of a claim directed to a device used in computer hard drive disks that required a “change in resistance by at least 10%” between two electrodes on the device. 687 F.3d at 1379–80. The background section of the patent explained that past efforts to “produce an adequate level of change in the [ ] resistance” had achieved only a 2.7% change. *Id.* at 1379. The Federal Circuit found the claims were not enabled. In relevant part, the court observed that the patent specification “only disclose[d] enough information to achieve an 11.8% resistive change,” even

---

<sup>15</sup> “Q. So if an antibody binds Factor IX or IXa and increases the procoagulant activity of Factor IXa by as much as Factor VIII does, that antibody is within the scope of the claim, correct? A. . . . yeah, I think it’s within the scope of the claim.”

<sup>16</sup> “Q. . . . [I]f it turned out that . . . there were an antibody that mirrored the activity of factor VIII itself, such an antibody would still be covered by these claims if it met the other limitations; correct? A. I would agree with that statement, yes.”

though the claims were construed to cover resistive changes “from 10% up to infinity.” *Id.* at 1383. The Federal Circuit further stated, “[t]he record contains no showing that the knowledge of [a skilled] artisan would permit, at the time of filing, achievement of the modern values above 600% without undue experimentation.” *Id.* at 1384. “Indeed,” the court observed, “it had taken “nearly twelve years of experimentation to actually reach those [modern] values.” *Id.* The same problem exists here.

Just as it took twelve years after the filing of the patent in *Magsil* for others to reach a 604% change in resistance, here, it took scientists at Chugai almost 10 years to discover the accused product emicizumab, which increases procoagulant activity by 10%. *See supra* § VI ¶ 28; Kitazawa Dep. Tr. at 241:12–20. The highest amount that any antibody disclosed in the specification is estimated to have increased the procoagulant activity of Factor IXa is by 3.75% of normal Factor VIII levels. *See supra* § VI ¶ 4. Both are far short of normal Factor VIII levels (at least 40%). *See id.* Although agreeing that an antibody that increases procoagulant activity by the same amount as would a normal level of Factor VIII would be within the scope of the claims, *see* Marasco Dep. Tr. at 236:12–18, Baxalta’s expert concedes “it would be difficult or indeed impossible to create” an antibody that increases the procoagulant activity by such an amount, Krishnaswamy Dep. Tr. at 212:18–213:7. A specification cannot adequately enable something that is admittedly impossible to accomplish. *See, e.g., Tr. of Boston Univ. v. Everlight Elecs. Co.*, 896 F.3d 1357, 1362 (Fed. Cir. 2018) (“We can safely conclude that the specification does not enable what the experts agree is physically impossible.”). But even as to the compounds within the realm of possibility, there is no enablement.

The stated object of the invention is “to provide a preparation for the treatment of blood coagulation disorders . . . .” ’590 patent, col. 2, ll. 25–28; *see also id.* at col. 9, ll. 25–36 (“The

present antibodies . . . are suitable for therapeutic use . . .”). And the primary utility disclosed in the ’590 patent for antibodies and antibody fragments that “increase[] the procoagulant activity of Factor IXa” is a therapeutic one. *See id.* col. 1, ll. 32–35; *id.*, col. 2, ll. 22–33; *id.*, col. 2, ll. 39–44; *id.*, col. 9, ll. 25–36; *id.*, col. 9, ll. 50–61.<sup>17</sup> But as discussed, the highest amount by which any antibody disclosed in the specification is estimated to have increased the procoagulant activity of Factor IXa (3.75%), would only be capable of moving a patient with hemophilia A classified as severe to a moderate classification. *See supra* § VI ¶ 4. It would not be capable of moving a patient with severe hemophilia to a mild classification. Although the experts debate whether such a small amount of procoagulant activity could be therapeutically useful for some patients, *see, e.g.*, Krishnaswamy Rpt. ¶¶ 115–118; Sheehan Rebuttal Rpt. ¶¶ 40–63, Baxalta acknowledges in the 20 years since the ’590 patent was filed, it has never brought to market a product embodying the ’590 patent’s invention, *see* Scheiflinger Dep. Tr. 48:25–49:11 (“no company has ever brought to market a monospecific antibody to Factor IXa to treat hemophilia”); *see also* Hr’g Tr. at 55:09–12 (counsel for Baxalta agreeing that none of the eleven examples in the patent were “developed into a therapeutic product”).

---

<sup>17</sup> After discussing the therapeutic utility of the claimed subject matter, the specification proposes other uses for the disclosed antibodies and antibody fragments, which only require that they bind to factor IX/IXa (not increase the procoagulant activity of factor IXa):

Moreover, the antibodies and antibody derivatives according to the invention may also be used for industrial applications, e.g. for the purification of factor IX/factor IXa by means of affinity chromatography, or as a component of detection methods (e.g. ELISA assays), or as an agent for identification of and interaction with functional domains of a target protein.

’590 patent, col. 9, ll. 50–56. Even if true that a stated purpose of the patent was the use of antibodies in industrial applications, that can hardly be used to show the enablement of what is clearly the primary purpose of the patent and indisputably covered by the claims—the treatment of hemophilia A.

What is more, the patent also claims therapeutic effectiveness in the presence of Factor VIII inhibitors (claim 2), necessary for the treatment of inhibitor patients. The only antibody that was subjected to the aPTT and measured in the presence of Factor VIII inhibitors was 193/AD3, *see supra* § VI ¶ 25, for which Baxalta's expert concedes the specification lacks "[s]ufficient information to accurately estimate the relevant rates of Factor Xa generation" in the chromogenic assay, Krishnaswamy Rpt. ¶ 122. Genentech's expert estimated, based on Figure 6A in the patent, that 193/AD3 (the only antibody tested in the presence of Factor VIII inhibitors) increases the level of procoagulant activity only at about 0.3–0.4% equivalent of Factor VIII activity, a marginal amount. *See supra* § VI ¶ 6.

Baxalta's expert now concedes that the patent's assertions that antibodies of the invention have therapeutic utility was merely "aspirational," Krishnaswamy Dep. Tr. at 42:05–45:14, and agrees that there is "not enough information conveyed in the patent to tell whether an antibody such as [198/A1] would have activity sufficient for clinical use," *id.* at 185:23–186:05.

Baxalta's only response to *MagSil* is that it is "factually inapposite" because it pertains only to claims to "a marginal improvement to a known quality," Pl.'s Opp'n at 34, and that a lower enablement standard should somehow apply to claims covering subject matters with previously unknown qualities, as Baxalta contends is the case here, *id.*; *see also* Hr'g Tr. 57:20–58:1–2.<sup>18</sup> Baxalta cites no caselaw for such a proposition, and the court does not read *MagSil* to be cabined in such a way. In *Plant Genetic Systems*, the Federal Circuit made clear that the enablement requirement is the same regardless of whether marginal or major advances are the subject of a

---

<sup>18</sup> "[The court]: There's no upper limit? [Counsel for Baxalta]: No, Your Honor, . . . this is not a quality that was known in advance."

patent. 315 F.3d at 1339–40 (rejecting as “not supported by precedents” an argument that a patent was entitled to a lower enablement requirement for a “pioneering” patent).

That the ’590 patent discloses a starting point for further research by disclosing a monospecific murine antibody that binds to Factor IX or IXa and increases the procoagulant activity of Factor IXa by a small amount is not sufficient enablement. *See Wyeth*, 720 F.3d at 1386 (determining that the specification provided “only a starting point for further iterative research in an unpredictable and poorly understood field”); *Storer v. Clark*, 860 F.3d 1340, 1350 (Fed. Cir. 2017) (“The specification need not recite textbook science, but it must be more than an invitation for further research.”).

### C. Structural Scope

*Third*, it is established that a claim is not enabled if it is structurally broad and there are insufficient working examples and guidance to enable the full scope of the structural limitations. *See, e.g., Idenix*, 941 F.3d at 1157–58. Here, claims 2–4 and 19–20 are necessarily within the scope of claim 1 because they are dependent claims. It is undisputed that the ’590 patent provides no working examples of two of the four *Markush*-group members in claims 3 and 20 (IgE and IgA), nor does the specification provide any guidance as to how one skilled in the art would alter the process disclosed in the patent or engineer antibodies to arrive at those isotypes. In fact, Baxalta’s expert testified that it would be exceedingly rare to discover antibodies of those isotypes for which there are no working examples. *See supra* § VI ¶ 3(a). Although the specification states that a class switch “may also be caused in a directed manner by means of genetic engineering methods” known in the prior art, ’590 patent, col. 6, ll. 41–45, the inventors of the ’590 patent did not perform such engineering or provide any specific guidance beyond reference to what was known in the prior art.

Nor are there any working examples of seven of the nine members of the *Markush* group in claim 4—“a chimeric antibody, a humanized antibody, . . . , a bispecific antibody, a diabody, and di-, oligo- or multimers thereof.” *See supra* § VI ¶ 3(b). Although a POSITA would have a general understanding of the process for modifying an antibody into these various formats, there is no specific direction as to the structure (*e.g.*, to what antigen the second arm of a bispecific antibody should bind),<sup>19</sup> and no assurance that, once the modifications are made, the antibody will retain the same functional qualities much less that making it bispecific would enhance its properties. *See id.* ¶ 26.

Of particular significance is the absence of any working examples of a humanized antibody (claim 19). While there are references to humanized antibodies in the list of antibody types known in the art, ’590 patent col. 6, ll. 15–19, 49–63, and a reference to prior-art humanization techniques, *see id.* at col. 7, l. 66–col. 8, l.4, the inventors never created a humanized antibody, provided no guidance in the specification as to how to create a humanized antibody that would exhibit the claimed function, and never determined whether humanizing an antibody (that would otherwise satisfy the claim limitations) would preserve its claimed procoagulant function, *see supra* § VI ¶ 27; Marasco Dep. Tr. at 120:13–20, 125:24–126:04. And because there is no way to predict whether, after humanization, an antibody or antibody fragment will retain its ability to bind to factor IX/IXa and increase the procoagulant activity of factor IXa, additional screening would be

---

<sup>19</sup> Although Baxalta’s expert opines that “a POSITA would know to identify a second binding specificity for the bispecific form of the claimed invention,” Pl.’s Opp’n, Ex. 12, ECF No. 424-13, Chang Rpt. ¶ 81, and that a “natural choice for a second binding specificity would be one of the two proteins associated with Factor IXa in the coagulation cascade: Factor VIII or Factor X,” *id.* at ¶ 82, an inventor of the ’590 patent admitted that, at the time of filing, they had not thought of or disclosed which antigens a bispecific antibody would bind, *see Kerschbaumer Dep. Tr.* at 19:459–461, and another Baxalta expert conceded that additional confirmatory testing would be necessary following modification of an antibody into a different format to ensure that the binding and activating functions of the antibody remained in place, Marasco Dep. Tr. at 127:24–128:25.

necessary after modification of the antibody. *See supra* § VI ¶ 27; Garcia Rpt. ¶¶ 84–85, 167. Baxalta’s expert Dr. Marasco wrote in a patent of his own, and confirmed at his deposition, that “humanizing an antibody is not as efficient a process as sometimes presented,” and that after humanization an antibody “frequently does not have the same effectiveness as the original murine antibody.” Marasco Dep. Tr. at 130:02–131:07.

#### D. Dependent Claims

So far, the court has been focused on claim 1, but the dependent claims fare no better. If anything, it is even clearer that the dependent claims are invalid given the dearth of working examples for the vast majority of what they claim as a matter of structure, as discussed *supra* § VIII.C. And the functional limitations of claim 1 apply equally to the dependent claims because they each claim the antibody or antibody fragment according to claim 1 and do not narrow the functional limitations of claim 1 in any way. *Alcon Rsch., Ltd. v. Apotex Inc.*, 687 F.3d 1362, 1367 (Fed. Cir. 2012) (“[B]ecause a dependent claim narrows the claim from which it depends, it must ‘incorporate . . . all the limitations of the claim to which it refers.’” (quotation omitted)). Accordingly, given the limited working examples for the structural limitations of the asserted dependent claims—including no working examples of antibodies that satisfy claim 19 (humanized antibodies), only examples of two of the four isotypes listed in claims 3 and 20, and no working examples of seven of the nine structural formats listed in claim 4—combined with the broad scope of the functional limitations under claim 1 and the lack of guidance in the specification, no reasonable jury could find the dependent claims are enabled.

#### E. Nonenablement of Emeticumab

Finally, it is significant that the patent does not remotely enable the accused antibody, emeticumab, which must fall within the scope of the claims to establish an infringement claim.



Emicizumab increases the procoagulant effect by approximately 10%<sup>20</sup>—an amount that has proven capable of reducing bleeding episodes in inhibitor patients by a clinically significant amount. *See* Smith Decl. ¶¶ 44, 49; Malackowski Rpt. at 34, 36. Yet, as discussed, the patent does not disclose an antibody that has procoagulant activity anywhere near that amount. None of the 11 disclosed antibodies in the specification increase the procoagulant activity of factor IXa more than 3.75%, and it is unknown whether that example would perform the same in the presence of Factor VIII inhibitors. *See supra* § VI ¶¶ 4, 6.

Moreover, key to emicizumab’s therapeutic effectiveness is its structure as a bispecific humanized antibody, *see* Sampei Article at 2, and as discussed, there is no working example of either a bispecific or humanized antibody in the specification of the ’590 patent, let alone an antibody that is both. Two inventors of the ’590 patent, Scheiflinger and Kerschbaumer, admitted they did not make a bispecific antibody, *see* Scheiflinger Dep. Tr. at 68:08–19; Kerschbaumer Tr. at 18:429–434, and Baxalta’s expert, Dr. Krishnaswamy, conceded that the patent’s “language” about antibodies of the invention have therapeutic utility was merely “aspirational,” Krishnaswamy Dep. Tr. at 42:05–45:14. Significantly, it took Chugai over ten years of multi-phased experimentation and the screening of tens of thousands of candidate compounds to discover emicizumab. *See supra* § VI ¶ 28.

Although Baxalta concedes that emicizumab is within the scope of the claims, it now argues that the court should not be concerned with the lack of enablement of emicizumab because

---

<sup>20</sup> Baxalta’s expert disputes the accuracy of this 10 percent figure, *see* Krishnaswamy Rpt ¶ 112, but that opinion is based on testing of emicizumab at a diluted concentration level and says nothing of the concentration level used in the treatment of hemophilia A. Moreover, the literature relied on by the same expert states that emicizumab at the treatment concentration level is “assumed to be equivalent to that of . . . 10% FVIII.” Uchida et al., *A first-in-human phase I study of ACE910, a novel factor VIII-mimetic bispecific antibody, in healthy subjects*, Blood 127(13):1633–1641 (2016) (cited as Ex. E in Krishnaswamy Rpt).

the high coagulant effect of emicizumab may be due to the fact that it is bispecific and binds Factor X with the other arm, whereas the asserted claims involve the arm that binds Factor IX. *See* Hr’g Tr. at 50:20–51:05. In that event, Baxalta suggests maybe a compound is not within the scope of the claims if the procoagulant effect is only caused by a bispecific antibody’s arm that binds to Factor X. *See id.* at 52:03–19.<sup>21</sup> This convoluted argument, offered for the first time at the Summary Judgment hearing, does nothing to show enablement. Baxalta convinced the Federal Circuit that bispecific antibodies are within the scope of the claims. It cannot now prevail by arguing that one bispecific antibody is perhaps not within the scope of the claims if its procoagulant activity results from its bispecific nature.

Past decisions have advised that patents should be awarded to the true inventor and that the enablement requirement serves an important purpose in this respect. *See Amgen Inc. v. Sanofi, Aventisub LLC*, 850 F. App’x 794, 796 (Fed. Cir. 2021) (denying rehearing en banc) (“One should not gain exclusivity over claimed subject matter without disclosing how to make and use it. And if one considers that one has invented a group of compositions defined by a genus but does not know enough to fully enable that genus, one would suppress innovation if one were able to claim such a broad genus, not enhance it.”); *see also J.E.M. Ag Supply*, 534 U.S. at 142 (identifying an enabling disclosure as the “*quid pro quo* of the right to exclude” (quoting *Kewanee Oil Co. v.*

---

<sup>21</sup> Counsel: “The claimed invention is about the bispecific antibody with the arm that binds Factor 9, 9A and that arm that exhibits the procoagulant activity on Factor 9, 9A, and it’s not necessary to enable or to fully describe even the portions of the . . . composition that are not part of the claim.”

Court: “[S]o there would be no infringement here if Hemlibra procoagulant activity resulted from the Factor 10 binding and not from the fact that it binds, the other chain binds to Factor 9A?”

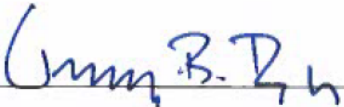
Counsel: “If the only procoagulant activity [] came from the binding of Factor 10 and there was no procoagulant activity as a result of the Factor [IX/IXA] arm, my understanding is that there would be no infringement.”

*Bicron Corp.*, 416 U.S. 470, 484 (1974)); *McRO*, 959 F.3d at 1099–100 (“The requirement of enablement . . . enforces the essential ‘*quid pro quo*’ of the patent bargain.” (quoting *AK Steel Corp. v. Sollac*, 344 F.3d 1234, 1244 (Fed. Cir. 2003))).

That is, the enablement requirement ensures that the entity that does the hard work to invent a useful compound is the recipient of the patent, not some earlier inventor who may have conceived of such a therapy or made the first step in research, but did not enable its ultimate production. See *Genentech*, 108 F.3d at 1366 (“Patent protection is granted in return for an enabling disclosure of an invention, not for vague intimations of general ideas that may or may not be workable.”); see also *Wyeth*, 720 F.3d at 1386 (finding that disclosing “only a starting point for further iterative research in an unpredictable and poorly understood field” does not constitute sufficient enablement). That is the situation here. The court cannot allow Baxalta to provide a starting point for further research and then claim “someone else’s solution to the problem.” *Genentech*, 108 F.3d at 1366.

### CONCLUSION

For the foregoing reasons, the court GRANTS Genentech’s motion for summary judgment of invalidity for lack of enablement and DENIES as moot Genentech’s motion for summary judgment in all other respects.

  
\_\_\_\_\_  
Honorable Timothy B. Dyk  
United States Circuit Judge, sitting by designation

**IN THE UNITED STATES DISTRICT COURT  
FOR THE DISTRICT OF DELAWARE**

BAXALTA INCORPORATED and  
BAXALTA GMBH,

Plaintiffs,

v.

GENENTECH, INC. and CHUGAI  
PHARMACEUTICAL CO., LTD.,

Defendants.

Civil Action No. 17-509-TBD

**ORDER**

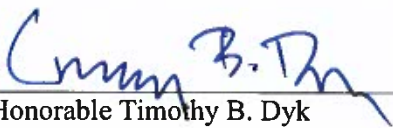
For reasons discussed in the accompanying Memorandum Opinion issued this 13th day of January, 2022,

**IT IS HEREBY ORDERED** that:

1. Genentech's Motion for Summary Judgment, ECF No. 407, is **GRANTED** on the ground that the asserted claims of the '590 patent are invalid for lack of enablement, and judgment is entered in favor of Genentech.
2. As the Memorandum Opinion was filed under seal, the parties shall meet and confer and shall on or before January 18, 2022, submit any proposed redactions. The parties are advised that redactions are strongly disfavored and proposed redactions must be accompanied by a showing of good cause. Thereafter, the court will issue a public version of its Memorandum Opinion.

This is a final appealable order.

**SO ORDERED.**

  
\_\_\_\_\_  
Honorable Timothy B. Dyk  
United States Circuit Judge, sitting by designation



US007033590B1

(12) **United States Patent**  
**Scheifflinger et al.**(10) **Patent No.:** **US 7,033,590 B1**  
(45) **Date of Patent:** **Apr. 25, 2006**(54) **FACTOR IX/FACTOR IXA ACTIVATING  
ANTIBODIES AND ANTIBODY  
DERIVATIVES**(75) Inventors: **Friedrich Scheifflinger**, Vienna (AT);  
**Randolf Kerschbaumer**, Vienna (AT);  
**Falko-Guenter Falkner**, Orth/Donau  
(AT); **Friedrich Dörner**, Vienna (AT);  
**Hans-Peter Schwarz**, Vienna (AT)(73) Assignee: **Baxter Aktiengesellschaft**, Vienna (AT)(\*) Notice: Subject to any disclaimer, the term of this  
patent is extended or adjusted under 35  
U.S.C. 154(b) by 462 days.(21) Appl. No.: **09/661,992**(22) Filed: **Sep. 14, 2000**(30) **Foreign Application Priority Data**

Sep. 14, 1999 (AT) ..... 1576/99

(51) **Int. Cl.****A61K 39/395** (2006.01)**A61K 38/04** (2006.01)**C12N 5/20** (2006.01)**C07K 16/00** (2006.01)**C07K 16/34** (2006.01)(52) **U.S. Cl.** ..... **424/145.1**; 435/326; 530/388.25;  
530/387.1; 530/327; 530/328; 530/389.3(58) **Field of Classification Search** ..... 530/387.3,  
530/388.25, 389.3, 327, 328; 424/133.1,  
424/145.1; 435/326

See application file for complete search history.

(56) **References Cited****U.S. PATENT DOCUMENTS**4,395,396 A 7/1983 Eibl et al.  
4,873,316 A 10/1989 Meade et al.  
5,932,706 A 8/1999 Mertens et al.  
6,391,299 B1 \* 5/2002 Blackburn et al.  
6,632,927 B1 \* 10/2003 Adair et al.**FOREIGN PATENT DOCUMENTS**WO WO95/13300 5/1995  
WO WO97/26010 7/1997  
WO WO99/01476 1/1999**OTHER PUBLICATIONS**Panka et al. Variable region framework differences result in  
decreased or increased affinity of variant anti-digoxin anti-  
bodies. *Proc Natl Acad Sci U S A.* 85(9):3080-3084, 1988.\*Rudikoff et al. Single amino acid substitution altering anti-  
gen-binding specificity. *Proc Natl Acad Sci U S A.*  
79(6):1979-1983, 1982.\*Ames, R.S. et al., *Conversion of Murina Fabs Isolated From  
a Combinatorial Phage Display Library to Full Length  
Immunoglobulins*, *J. Immunol. Methods*, pp. 177-186  
(1995).Bajaj, S.P. et al., *A Monoclonal Antibody to Factor IX That  
Inhibits the Factor VIII:Ca Potentiation of Factor X Acti-  
vation*, *The Journal of Biological Chemistry*, 260(21), pp.  
11574-11580 (1985).Bessos, H., et al., *The Characterization of a Panel of  
Monoclonal Antibodies to Human Coagulation Factor IX*,  
*Thrombosis Research*, 40, pp. 863-867 (1985).Cao, Y. et al., *Bispecific Antibodies as Novel Bioconjugates*,  
*Bioconjugate Chemistry*, 9(6), pp. 635-644 (1998).Cohen, F.E., et al., *The Combinatorial Approach, Protein  
Structure Prediction—A Practical Approach* (Ed. M.J.E.  
Sternberg), Oxford University Press, Ch. 9, pp. 207-227  
(1996).Engelhardt, O., et al., *Two Step Cloning of Antibody Variable  
Domains in a Phage Display Vector*, *Biotechniques*, 17, p.  
44-46 (1994).Esser, C., et al., *Immunoglobulin Class Switching: Molecu-  
lar and Cellular Analysis*, *Annu. Rev. Immunol.*, 8, p.  
717-735 (1990).Evan, G.I., et al., *Isolation of Monoclonal Antibodies Spe-  
cific for Human c-myc Proto-Oncogene Product*, *Mol. Cell.  
Biol.*, 5(12), p. 3610-3616 (1985).Fay, P.J., et al., *Factor VIII A2 Subunit Residues 558-565  
Represent a Factor IXa Interactive Site*, *Journal of Biologi-  
cal Chemistry*, 269(32), p. 20522-20527 (1994).Frazier, D., et al., *Mapping of Monoclonal Antibodies to  
Human Factor IX*, *Blood*, 74(3), p. 971-977 (1989).Gao, C., et al., *Making Artificial Antibodies: A Format for  
Phage Display of Combinatorial Heterodimeric Arrays*,  
*Proc. Natl. Acad. Sci.*, 96, p. 6025-6030 (1999).Grassy, G., et al., *Computer-Assisted Rational Design of  
Immunosuppressive Compounds*, *Nature Biotechnology*, 16,  
p. 748-752 (1998).Greer, J., et al., *Application of the Three-Dimensional  
Structures of Protein Target Molecules in Structure-Based  
Drug Design*, *Journal of Medicinal Chemistry*, 37(8), p.  
1035-1054 (1994).Harlow, E., et al., 2. *Antibody Molecules, Antibodies—A  
Laboratory Manual*; pp. 7-22 (1988).Harlow, E., et al., 3. *Antibody-Antigen Interactions, Anti-  
bodies—A Laboratory Manual*; p. 23-35 (1988).Harlow, E., et al., 6. *Monoclonal Antibodies, Antibodies—A  
Laboratory Manual*; p. 139-243 (1988).Hochuli, E., et al., *Genetic Approach to Facilitate Purifica-  
tion of Recombinant Proteins with a Novel Metal Chelate  
Adsorbent*, *Biotechnology*, 6, p. 1321-1325 (1988).Huston, J.S., et al., *Medical Applications of Single-Chain  
Antibodies*, *Intern. Rev. Immunol.*, 10, p. 195-217 (1993).

(Continued)

Primary Examiner—Christina Chan

Assistant Examiner—Maher Haddad

(74) Attorney, Agent, or Firm—Townsend and Townsend  
and Crew LLP

(57)

**ABSTRACT**An antibody or antibody derivative against factor  
IX/activated factor IX (FIXa) which increases the proco-  
agulant activity of FIXa.**22 Claims, 61 Drawing Sheets**

## US 7,033,590 B1

Page 2

## OTHER PUBLICATIONS

- Jones, D.T., et al., *Protein Folds and Their Recognition from Sequence, Protein Structure Prediction—A Practical Approach* (Ed. M.J.E. Sternberg), Oxford University Press, Ch. 8, p. 174–206 (1996).
- Jones, P.T., et al., *Replacing the Complementarity—Determining Regions in a Human Antibody with Those from a Mouse*, *Nature*, 321, p. 522–525 (1986).
- Jorquera, J.I., et al., *Synthetic Peptides Derived from Residues 698 to 710 of Factor VIII Inhibit Factor IXa Activity*, *Circulation*, 86, Abstract No. 2725, p. 1–685 (1992).
- Karpen, M.E., et al., *Modelling Protein Conformation by Molecular Mechanics and Dynamics, Protein Structure Prediction—A Practical Approach* (Ed. M.J.E. Sternberg), Oxford University Press, Ch. 10, p. 229–261 (1996).
- Kemp, D.S., *Peptidomimetics and the Template Approach to Nucleation of  $\beta$ -sheets and  $\alpha$ -helices in Peptides*, *TIBTECH*, p. 249–255 (1990).
- Kerschbaumer, R.J., et al., *pDAP2: A Vector for Construction of Alkaline Phosphatase Fusion-Proteins*, *Immunotechnology*, 2, p. 145–150 (1996).
- Kerschbaumer, R.J. et al., *Single-Chain Fv Fusion Proteins Suitable as Coating and Detecting Reagents in a Double Antibody Sandwich Enzyme-Linked Immunosorbent Assay*, *Analytical Biochemistry*, 249, p. 219–227 (1997).
- Lane, R.D., *A Short-Duration Polyethylene Glycol Fusion Technique for Increasing Production of Monoclonal Antibody-Secreting Hybridomas*, *Journal of Immunological Methods*, 81, p. 223–227 (1985).
- Lenting, P.J., et al., *The Sequence Glu<sup>1811</sup>–Lys<sup>1818</sup> of Human Blood Coagulation Factor VIII Comprises a Binding Site for Activated Factor IX*, *Journal of Biological Chemistry*, 271(4), p. 1935–1940 (1996).
- Liles, D.K., et al., *The Factor VIII Peptide Consisting of Amino Acids 698 to 712 Enhances Factor IXa Cleavage of Factor X*, *Blood*, 90(1), Abstract No. 2054, p. 463a (1997).
- Lin, H-F., et al., *A Coagulation Factor IX-Deficient Mouse Model for Human Hemophilia B*, *Blood*, 90(10), p. 3962–3966 (1997).
- Malik, P., et al., *Multiple Display of Foreign Peptide Epitopes on Filamentous Bacteriophage Virions, Phage Display of Peptides and Proteins* (Ed. B. K. Kay et al.), Academic Press, p. 127–139 (1996).
- Mann, K.G., et al., *Surface-Dependent Reactions of the Vitamin K-Dependent Enzyme Complexes*, *Blood*, 76(1), p. 1–16 (1990).
- Mikaelsson, M., et al., *Standardization of VIII:C Assays: A Manufacturer's View*, *Scandinavian Journal of Haematology* (Ed. Nilsson et al.), 33, p. 79–86 (1984).
- Nilsson, I.M. et al., *Induction of Split Tolerance and Clinical Cure in High-Responding Hemophiliacs with Factor IX Antibodies*, *Proc. Natl. Acad. Sci. USA*, 83, p. 9169–9173 (1986).
- Persic, L., et al., *An Integrated Vector System For The Eukaryotic Expression of Antibodies of Their Fragments After Selection From Phase Display Libraries*, *Genes*, p. 9–18 (1997).
- Pluckthun, A., et al., *New Protein Engineering Approaches to Multivalent and Bispecific Antibody Fragments*, *Immunotechnology*, 3, p. 83–105 (1997).
- Raag, R., et al., *Single-Chain Fvs*, *FASEB Journal*, 9(1), pp. 73–80 (1995).
- Rees, A.R., et al., *Antibody Combining Sites: Structure and Prediction, Protein Structure Prediction—A Practical Approach* (Ed. M.J.E. Sternberg), Oxford University Press, Ch. 7, p. 141–172 (1996).
- Roitt, I.M., et al., *Molecules which Recognize Antigen*, *Immunology*, 2<sup>nd</sup> Edition, p. 5.1–5.11 (1989).
- Sadler, J.E., et al., *Hemophilia A, Hemophilia B, and von Willebrand's Disease, The Molecular Basis of Blood Diseases* (Ed. G. Stamatoyannopoulos et al.), p. 575–630 (1987).
- Vaughan, T.J., et al., *Human Antibodies By Design*, *Nature Biotechnology*, p. 535–539 (1998).
- Winter, G., et al., *Making Antibodies by Phage Display Technology*, *Annu. Rev. Immunol.*, 12, p. 433–455 (1994).
- Zhong, D., et al., *Some Human Inhibitor Antibodies Interface with Factor VIII Binding to Factor IX*, *Blood*, 92(1), p. 136–142 (1998).

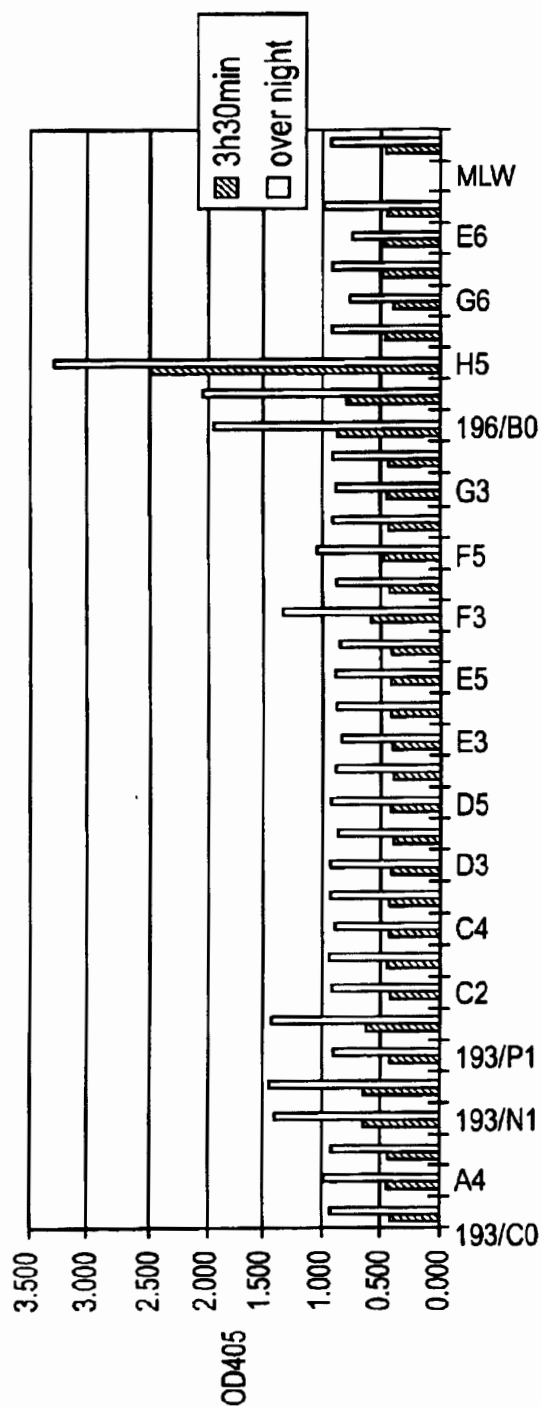
\* cited by examiner

U.S. Patent

Apr. 25, 2006

Sheet 1 of 61

US 7,033,590 B1



clone #

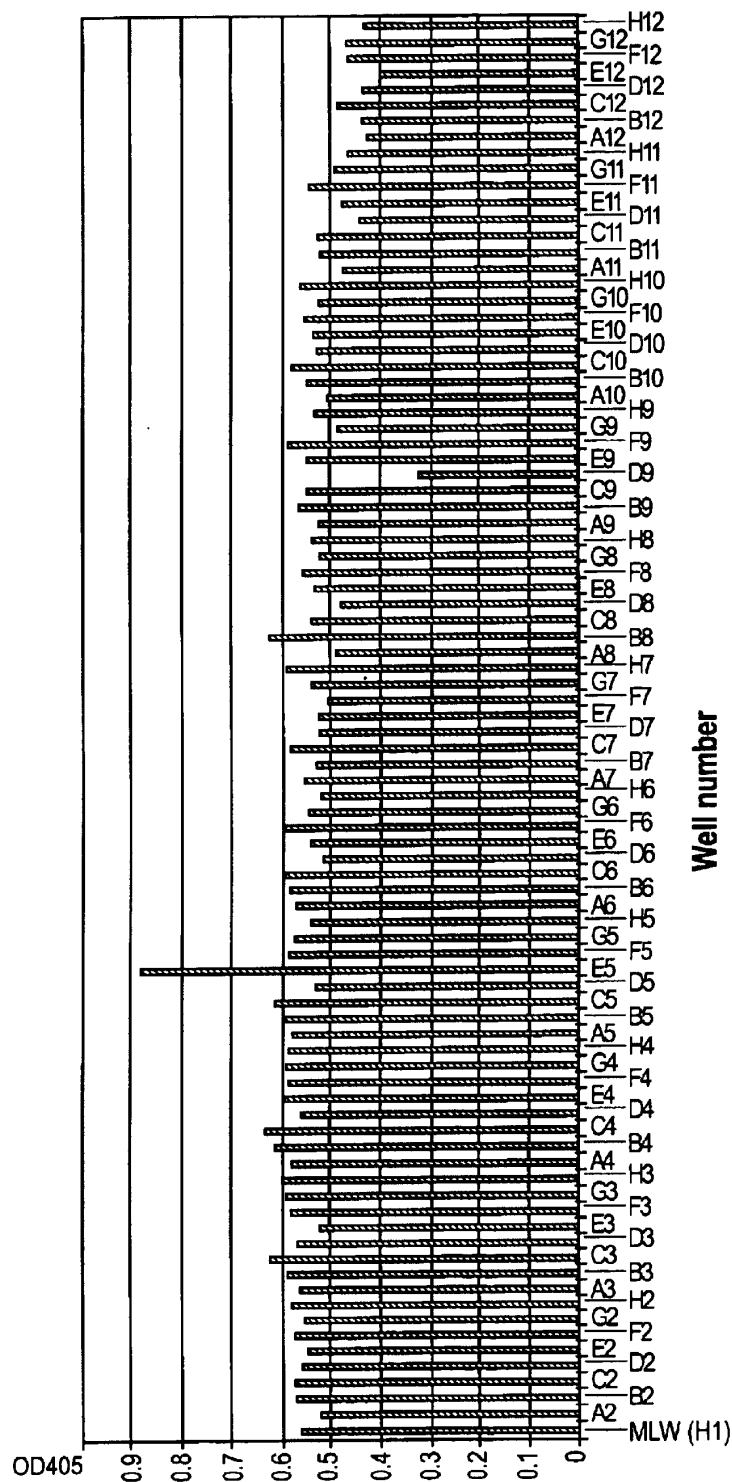
FIG. 1

U.S. Patent

Apr. 25, 2006

Sheet 2 of 61

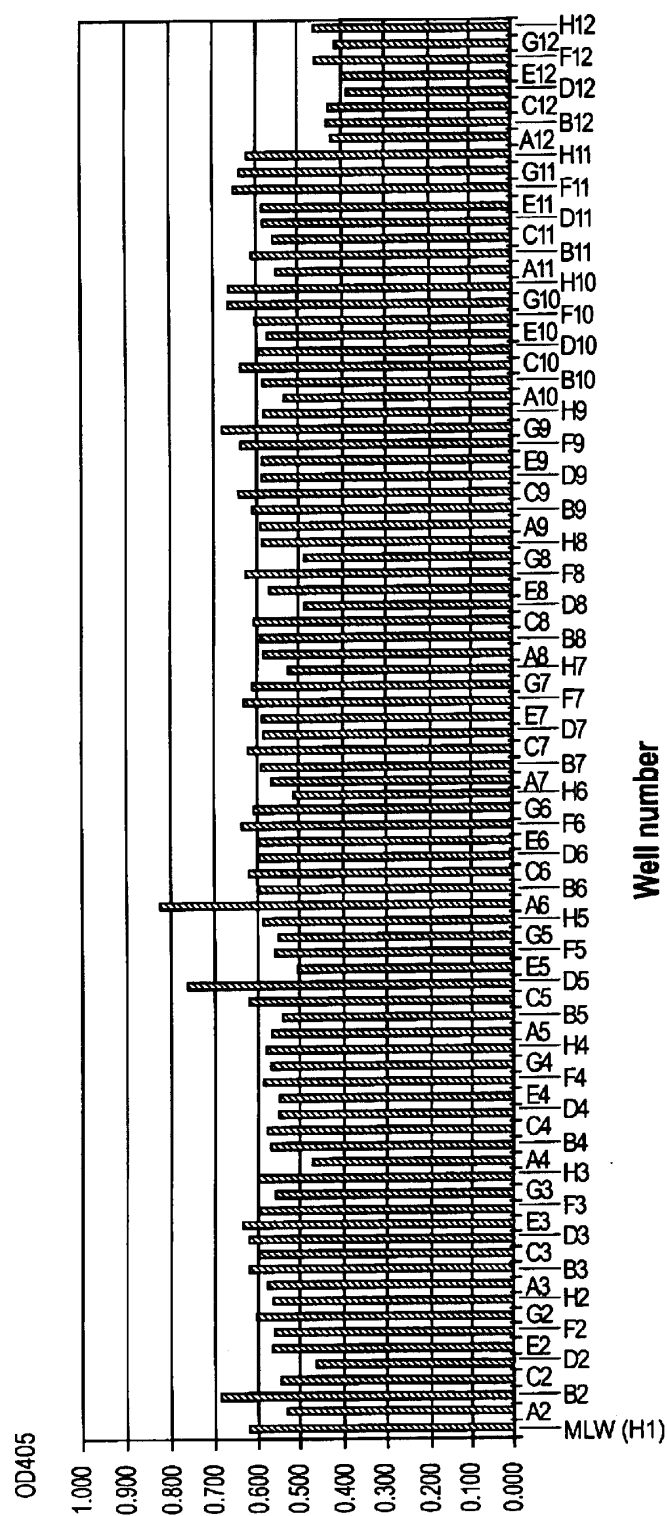
US 7,033,590 B1



Well number

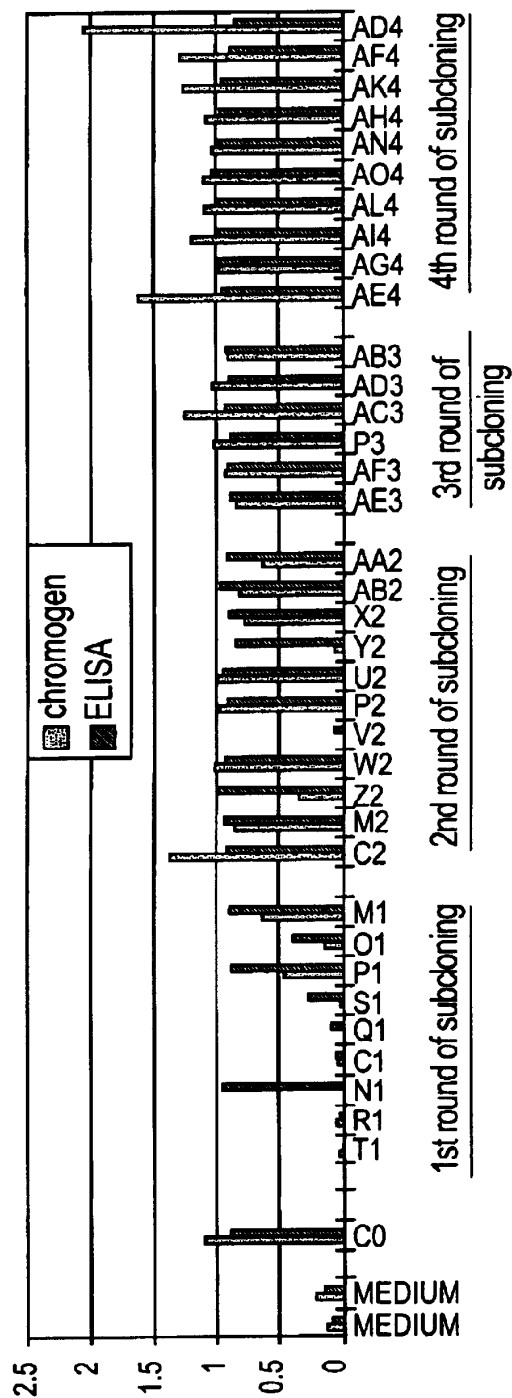
FIG. 2





Well number

FIG. 3

**FIG. 4**

U.S. Patent

Apr. 25, 2006

Sheet 5 of 61

US 7,033,590 B1

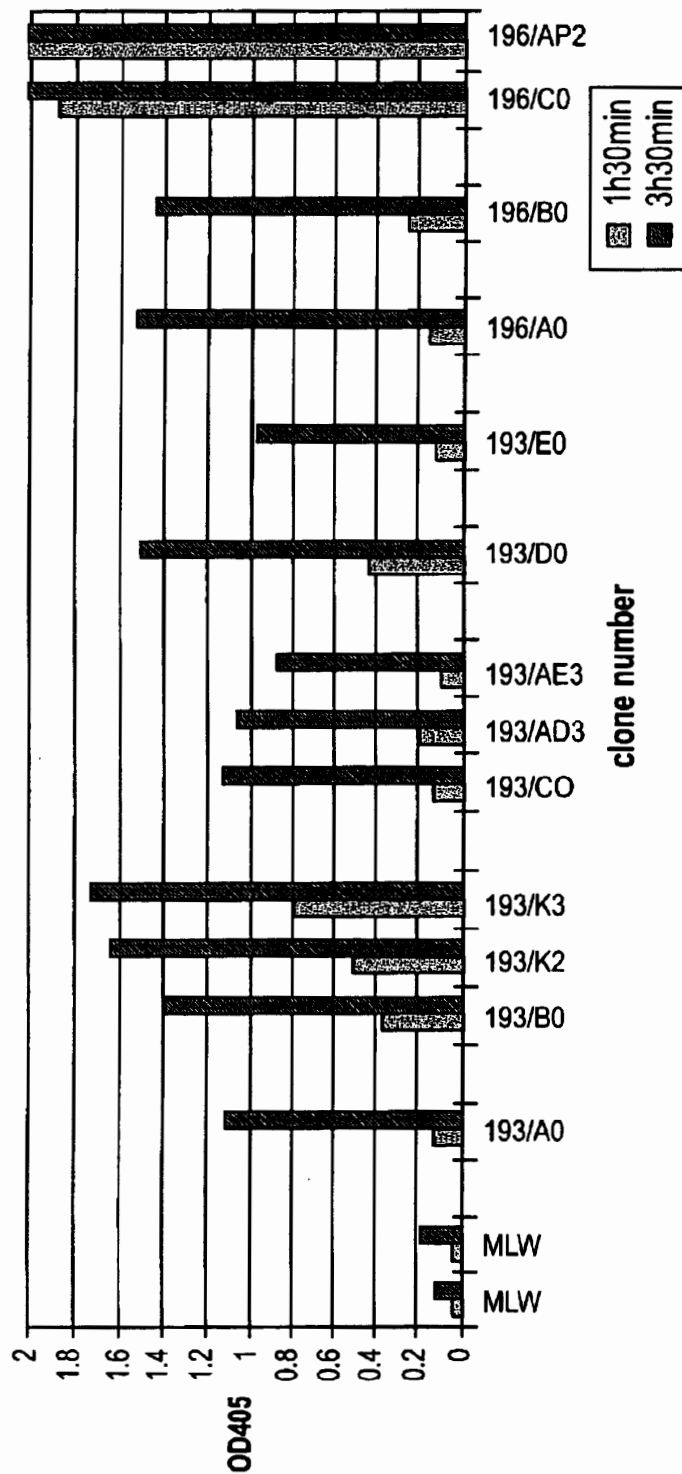


FIG. 5

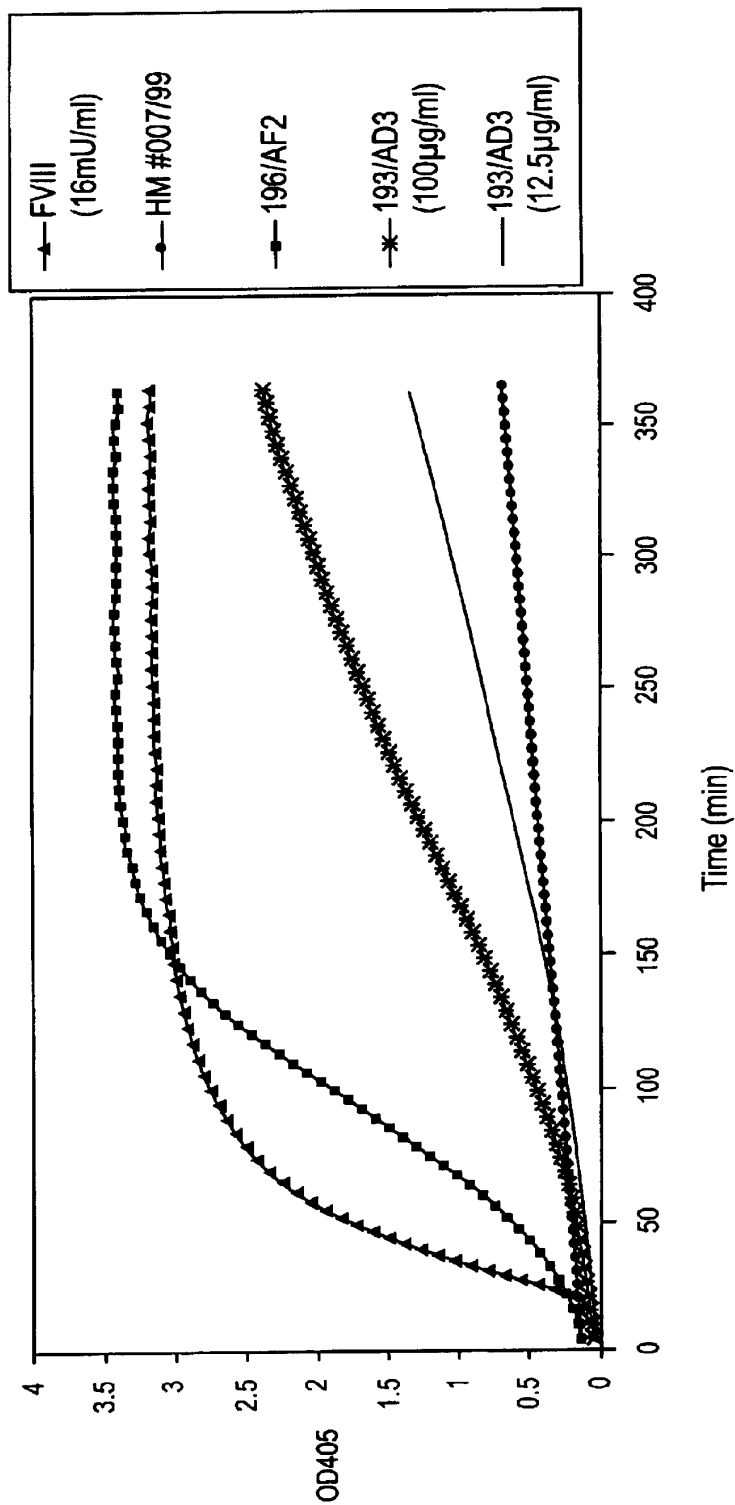
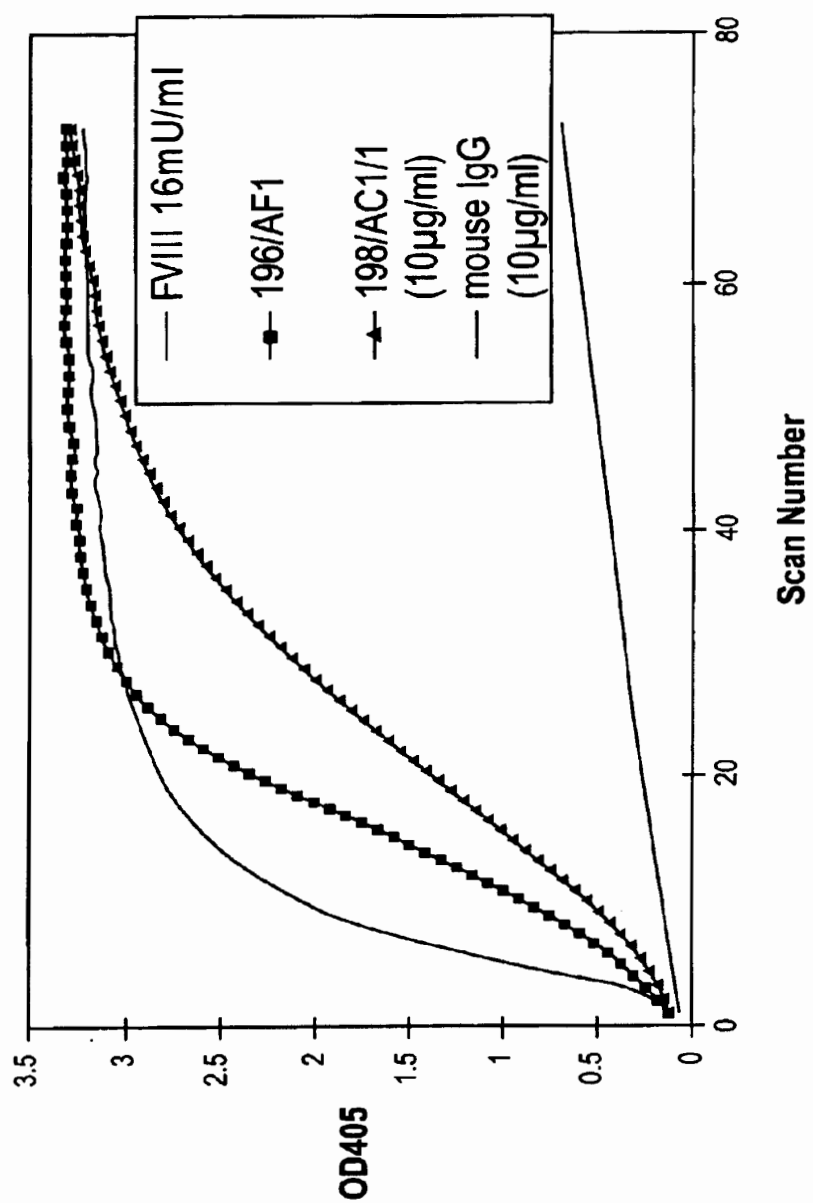


FIG. 6A

**FIG. 6B**

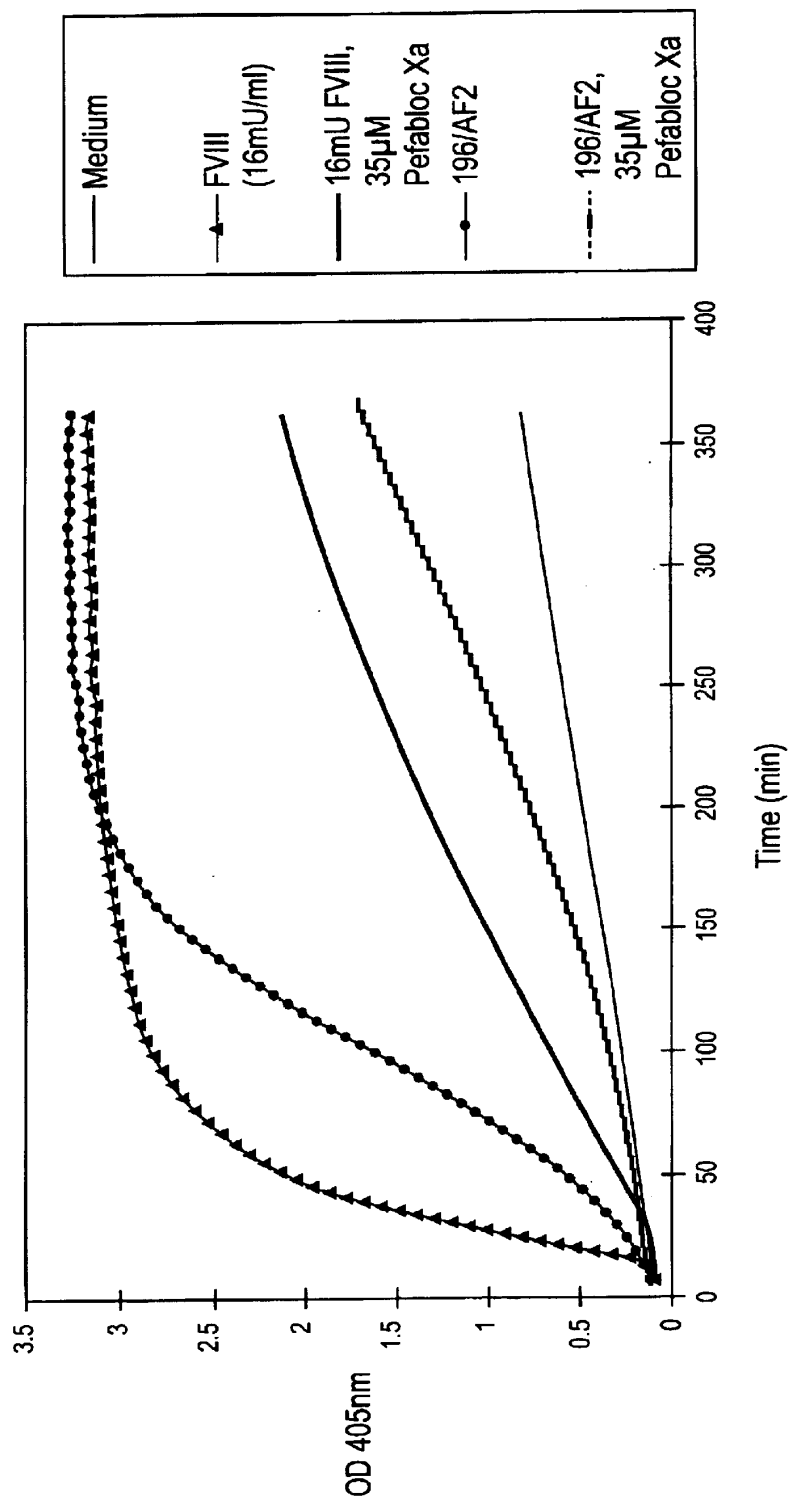


FIG. 7A

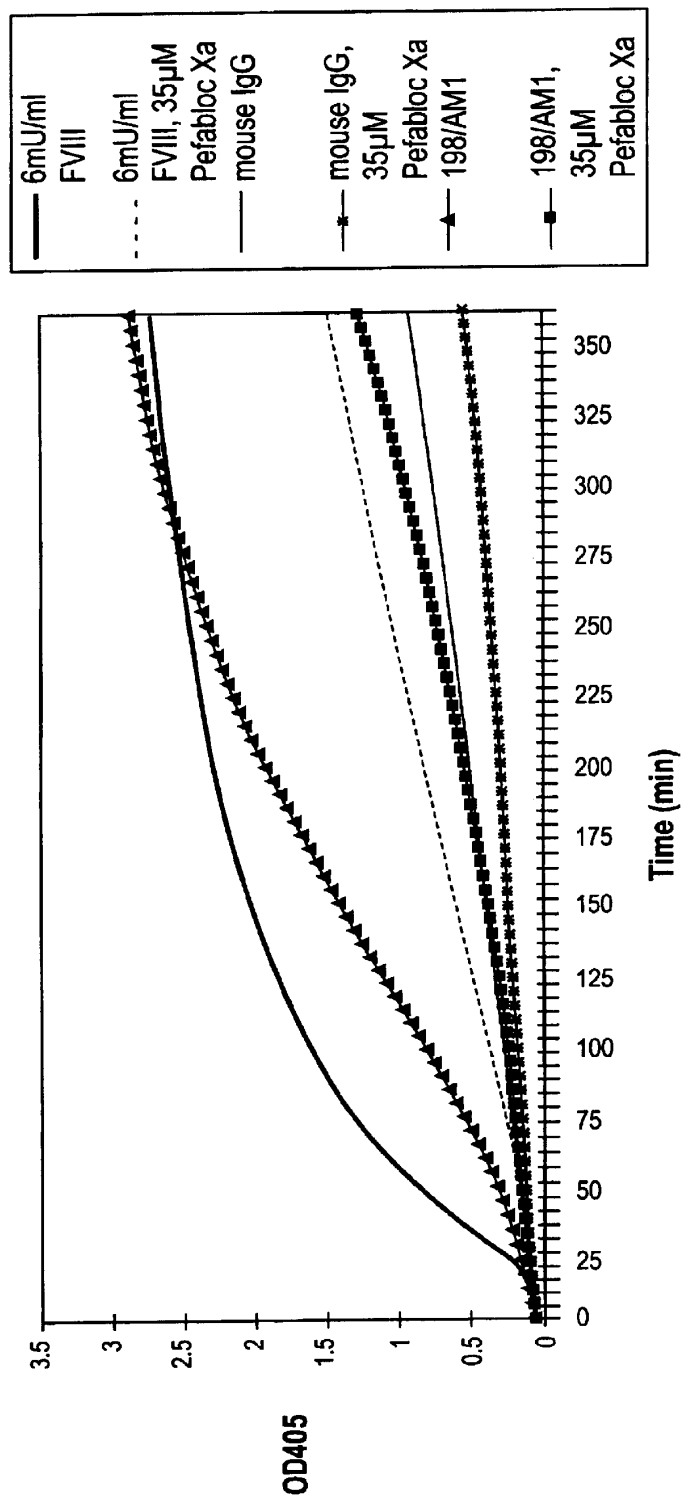


FIG. 7B

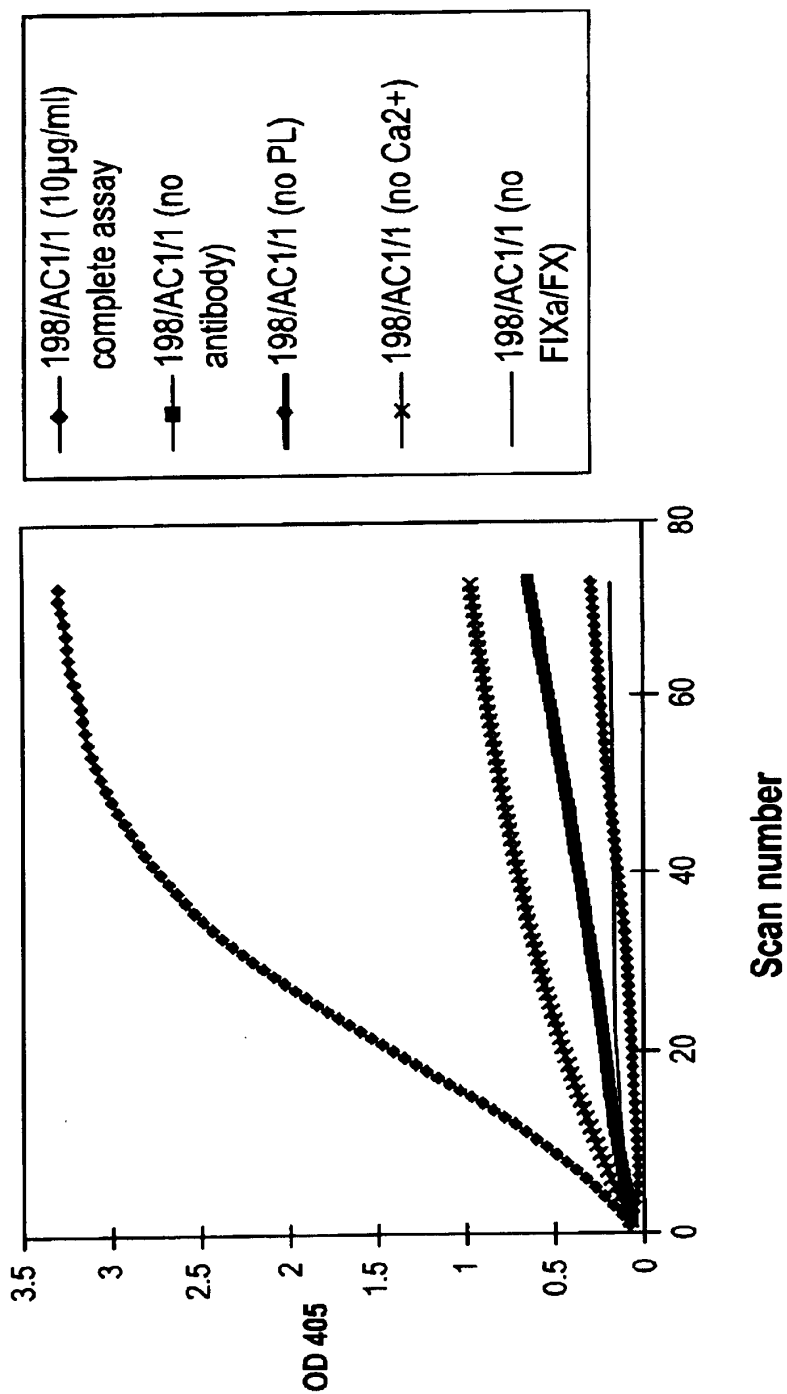
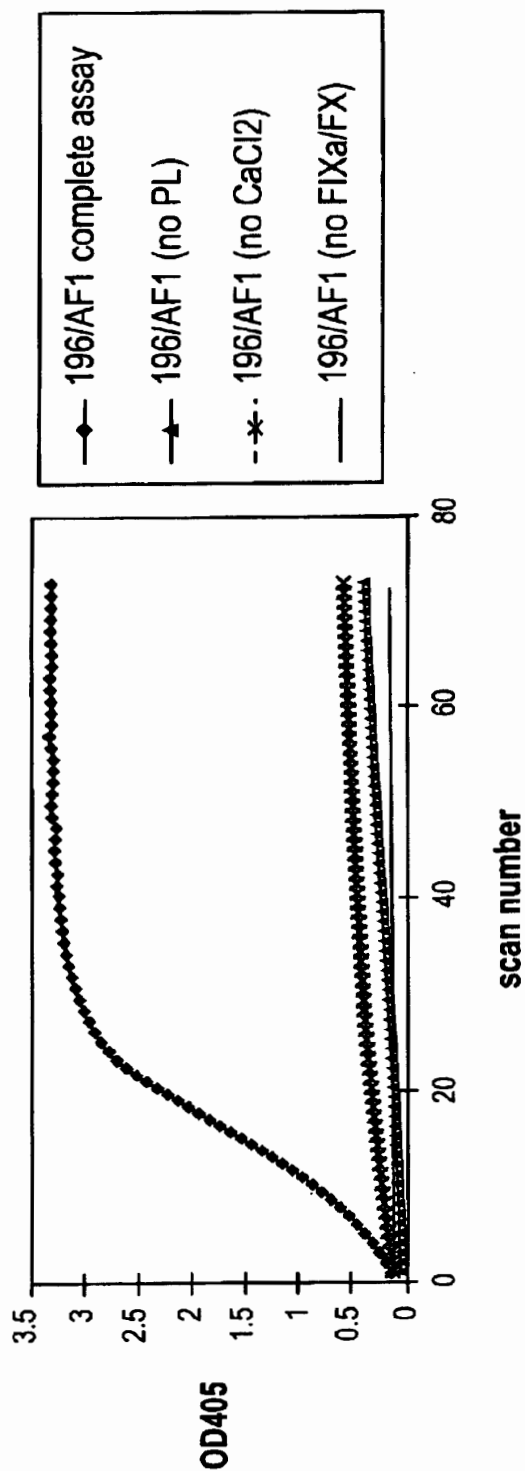


FIG. 8A



**FIG. 8B**

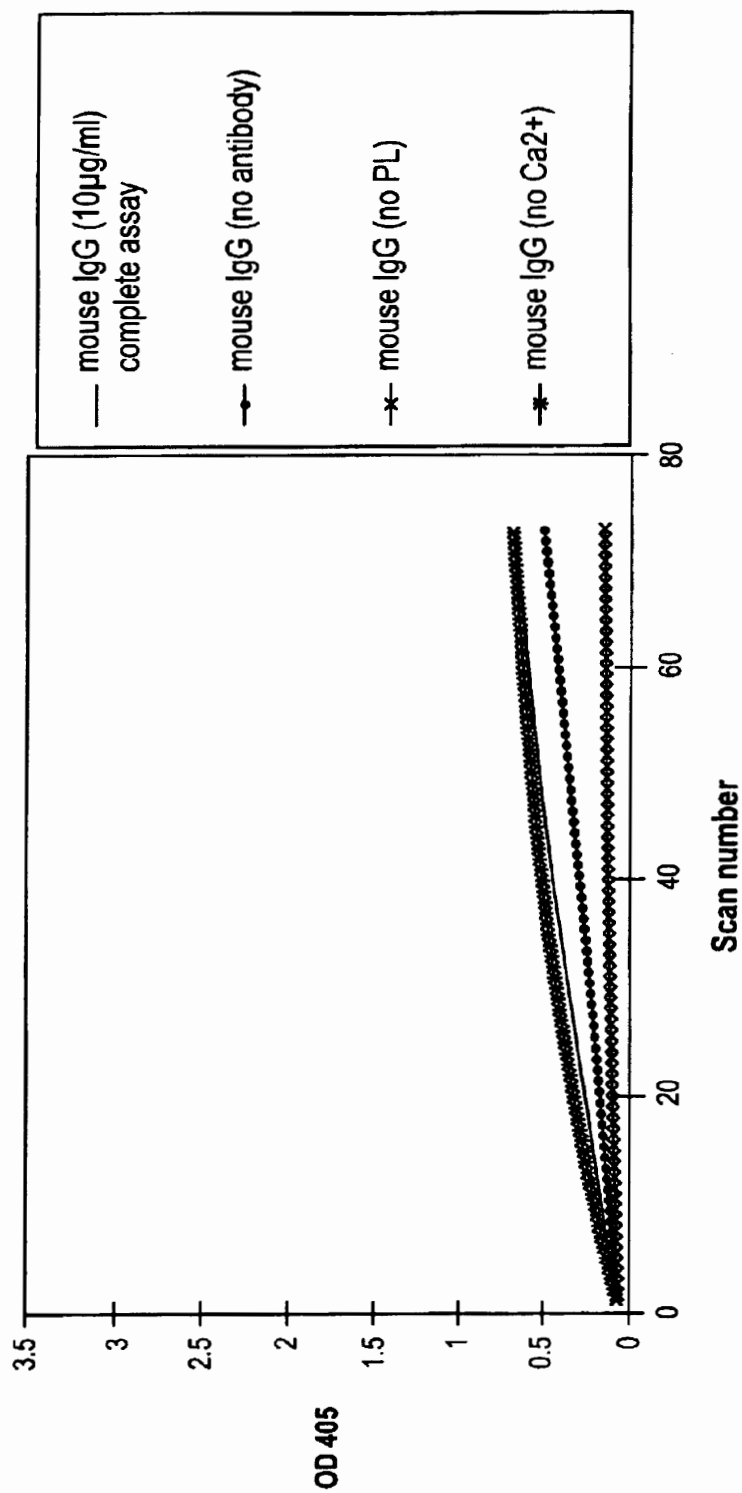


FIG. 8C

U.S. Patent

Apr. 25, 2006

Sheet 13 of 61

US 7,033,590 B1

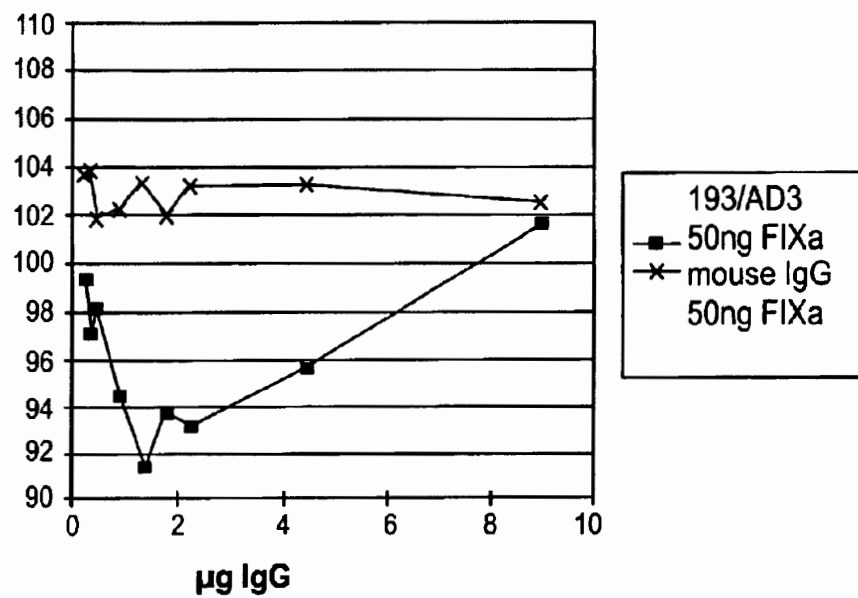


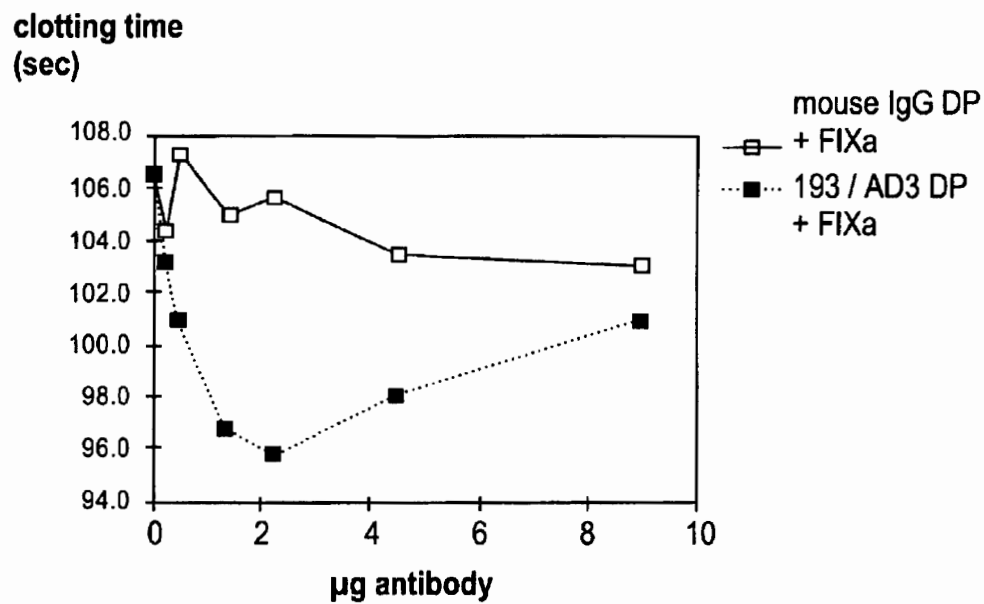
FIG. 9

U.S. Patent

Apr. 25, 2006

Sheet 14 of 61

US 7,033,590 B1



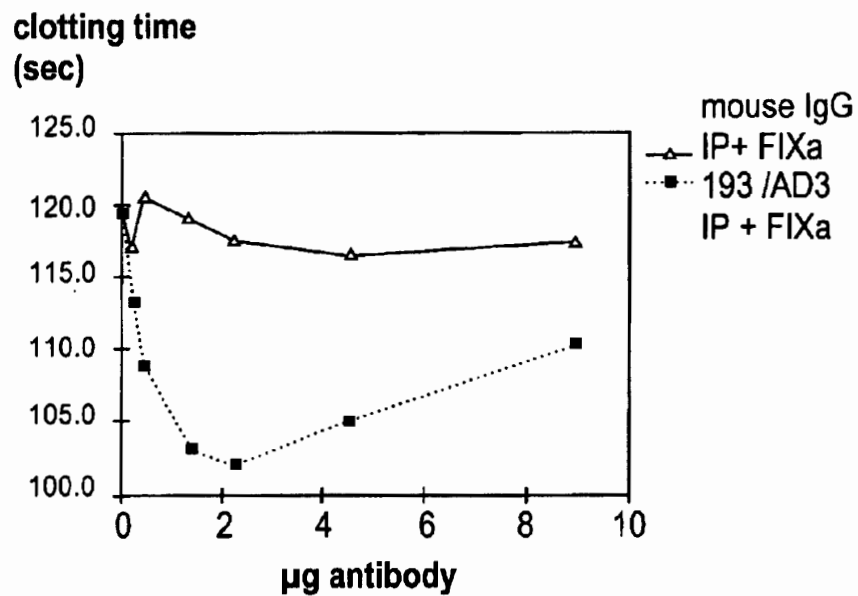
**FIG. 10A**

U.S. Patent

Apr. 25, 2006

Sheet 15 of 61

US 7,033,590 B1



**FIG. 10B**

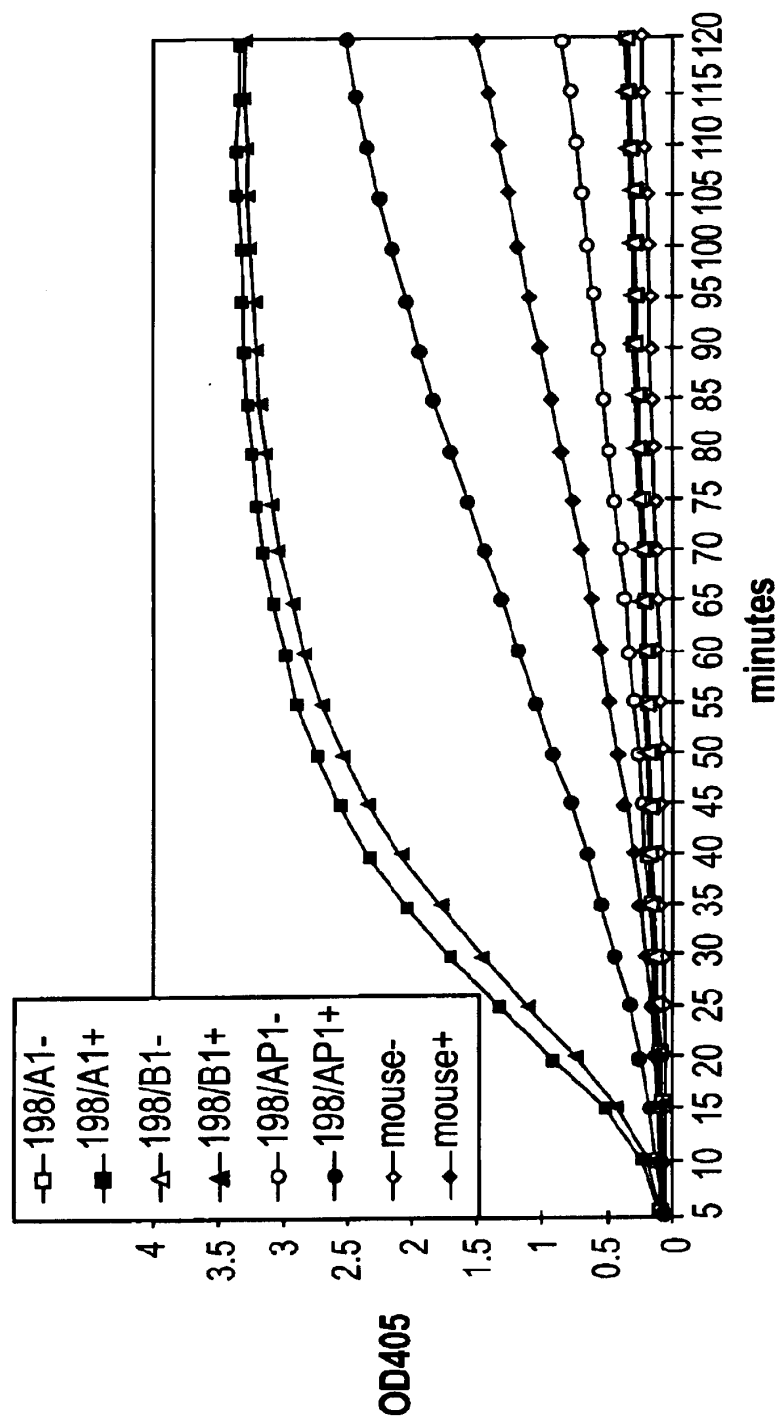


FIG. 11

Mouse  $V_H$  back primers (containing SfiI-site):

VH1BACK-SfiI 5' C ATG CCA TGA CTC GCG GCC CAG CCG GCC ATG GCC SAG GTS MAR CTG CAG  
SAG TCW GG 3' (SEQ.ID.NO. 50)

VH1BACKSfi 5' GTC CTC GCA ACT GCG GCC CAG CCG GCC ATG GCC GAG GTG CAG CTT CAG GAG TCA  
GG 3' (SEQ.ID.NO. 51)

VH2BACKSfi 5' GTC CTC GCA ACT GCG GCC CAG CCG GCC ATG GCC GAT GTG CAG CTT CAG GAG TCR  
GG 3' (SEQ.ID.NO. 52)

VH3BACKSfi 5' GTC CTC GCA ACT GCG GCC CAG CCG GCC ATG GCC CAG GTG CAG CTG AAG SAG TCA  
GG 3' (SEQ.ID.NO. 53)

VH4/6BACKSfi 5' GTC CTC GCA ACT GCG GCC CAG CCG GCC ATG GCC GAG GTY CAG CTG CAR CAR TCT  
GG 3' (SEQ.ID.NO. 54)

VH5/9BACKSfi 5' GTC CTC GCA ACT GCG GCC CAG CCG GCC ATG GCC CAG GTY CAR CTG CAG CAG YCT  
GG 3' (SEQ.ID.NO. 55)

VH7BACKSfi 5' GTC CTC GCA ACT GCG GCC CAG CCG GCC ATG GCC GAR GTG AAG CTG GTG GAR TCT  
GG 3' (SEQ.ID.NO. 56)

VH8BACKSfi 5' GTC CTC GCA ACT GCG GCC CAG CCG GCC ATG GCC GAG GTT CAG CTT CAG CAG TCT  
GG 3' (SEQ.ID.NO. 57)

VH10BACKSfi 5' GTC CTC GCA ACT GCG GCC CAG CCG GCC ATG GCC GAA GTG CAG CTG KTG GAG WCT  
GG 3' (SEQ.ID.NO. 58)

VH11BACKSfi 5' GTC CTC GCA ACT GCG GCC CAG CCG GCC ATG GCC CAG ATC CAG TTG CTG CAG TCT  
GG 3' (SEQ.ID.NO. 59)

FIG. 12-1

Mouse J<sub>H</sub> forward primers (containing 1/2 linker-sequence and AscI-site) :

VH1FOR2LiAsc	5' ACC GCC AGA GGC GCG CCC ACC TGA ACC GCC TCC ACC TGA GGA GAC GGT GAC CGT GGT CCC TTG GCC CC 3' (SEQ.ID.NO. 60)
JH1FORLiAsc	5' ACC GCC AGA GGC GCG CCC ACC TGA ACC GCC TCC ACC TGA GGA GAC GGT GAC CGT GGT CCC 3' (SEQ.ID.NO. 61)
JH2FORLiAsc	5' ACC GCC AGA GGC GCG CCC ACC TGA ACC GCC TCC ACC TGA GGA GAC TGT GAG AGT GGT GCC 3' (SEQ.ID.NO. 62)
JH3FORLiAsc	5' ACC GCC AGA GGC GCG CCC ACC TGA ACC GCC TCC ACC TGC AGA GAC AGT GAC CAG AGT CCC 3' (SEQ.ID.NO. 63)
JH4FORLiAsc	5' ACC GCC AGA GGC GCG CCC ACC TGA ACC GCC TCC ACC TGA GGA GAC GGT GAC TGA GGT TCC 3' (SEQ.ID.NO. 64)

---

IUPAC-Code: M=A/C, W=A/T, R=A/G, Y=C/T, S=C/G, K=G/T, H=A/C/T, D=A/G/T, V=A/C/G, B=T/C/G.

**FIG. 12-2**



**Primers for cloning mouse  $V_k$  genes**  
**Mouse  $V_k$  back primers (containing *AscI*-site and 1/2 linker-sequence):**

VK2BACK-LiAscI	5' GGT TCA GAT GGG CGC GCC TCT GGC GGT GGC GGA TCG GAC ATT GAG CTC ACC CAG TCT CCA 3' (SEQ.ID.NO. 65)
VK1BACKLi Asc	5' GGT TCA GAT GGG CGC GCC TCT GGC GGT GGC GGA TCG GAC ATT GTG ATG WCA CAG TCT CC 3' (SEQ.ID.NO. 66)
VK2BACKLi Asc	5' GGT TCA GAT GGG CGC GCC TCT GGC GGT GGC GGA TCG GAT GTT KTG ATG ACC CAA ACT CC 3' (SEQ.ID.NO. 67)
VK3BACKLi Asc	5' GGT TCA GAT GGG CGC GCC TCT GGC GGT GGC GGA TCG GAT ATT GTG ATR ACB CAG GCW GC 3' (SEQ.ID.NO. 68)
VK4BACKLi Asc	5' GGT TCA GAT GGG CGC GCC TCT GGC GGT GGC GGA TCG GAC ATT GTG CTG ACM CAR TCT GC 3' (SEQ.ID.NO. 69)
VK5BACKLi Asc	5' GGT TCA GAT GGG CGC GCC TCT GGC GGT GGC GGA TCG SAA AWT GTK CTC ACC CAG TCT CC 3' (SEQ.ID.NO. 70)
VK6BACKLi Asc	5' GGT TCA GAT GGG CGC GCC TCT GGC GGT GGC GGA TCG GAY ATY VWG ATG ACM CAG WCT CC 3' (SEQ.ID.NO. 71)
VK7BACKLi Asc	5' GGT TCA GAT GGG CGC GCC TCT GGC GGT GGC GGA TCG CAA ATT GTT CTC ACC CAG TCT CC 3' (SEQ.ID.NO. 72)
VK8BACKLi Asc	5' GGT TCA GAT GGG CGC GCC TCT GGC GGT GGC GGA TCG TCA TTA TTG CAG GTG CTT GTG GG 3' (SEQ.ID.NO. 73)

**FIG. 13-1**

*Mouse J<sub>K</sub> forward primers (containing NotI-site):*

JK1NOT10	5' GAG TCA TTC TGC GGC CGC CCG TTT GAT TTC CAG CTT GGT GCC 3' (SEQ.ID.NO. 74)
JK2NOT10	5' GAG TCA TTC TGC GGC CGC CCG TTT TAT TTC CAG CTT GGT CCC 3' (SEQ.ID.NO. 75)
JK3NOT10	5' GAG TCA TTC TGC GGC CGC CCG TTT TAT TTC CAG TCT GGT CCC 3' (SEQ.ID.NO. 76)
JK4NOT10	5' GAG TCA TTC TGC GGC CGC CCG TTT TAT TTC CAA CTT TGT CCC 3' (SEQ.ID.NO. 77)
JK5NOT10	5' GAG TCA TTC TGC GGC CGC CCG TTT CAG CTC CAG CTT GGT CCC 3' (SEQ.ID.NO. 78)

---

IUPAC-Code: K=G/T, M=A/C, W=A/T, R=A/G, Y=C/T, S=C/G, H=A/C/T, D=A/G/T, V=A/C/G, B=T/C/G.

**FIG. 13-2**

U.S. Patent

Apr. 25, 2006

Sheet 21 of 61

US 7,033,590 B1

## VH

```

+1   E   V   K   L   V   E   S   G   P   E   L   K   K   P   G
1   GAG GTG AAG CTG GTG GAG TCT GGA CCT GAG CTG AAG AAG CCT GGA

+1   E   T   V   K   I   S   C   K   A   S   G   Y   I   F   T
46  GAG ACA GTC AAG ATC TCC TGC AAG GCT TCT GGG TAT ATC TTC ACA

+1   N   Y   G   M   N   W   V   K   Q   A   P   G   K   G   L
91  AAC TAT GGA ATG AAC TGG GTG AAG CAG GCT CCA GGA AAG GGT TTA

+1   K   W   M   G   W   I   N   T   Y   T   G   E   P   T   Y
136 AAG TGG ATG GGC TGG ATA AAC ACC TAC ACT GGA GAG CCA ACA TAT

+1   A   D   D   F   K   G   R   F   A   F   S   L   E   T   S
181 GCT GAT GAC TTC AAG GGA CGG TTT GCC TTC TCT TTG GAA ACC TCT

+1   A   S   T   A   Y   L   Q   I   N   N   L   K   N   E   D
226 GCC AGC ACT GCC TAT TTG CAG ATC AAC AAC CTC AAA AAT GAG GAC

+1   T   A   T   Y   F   C   A   L   Y   G   N   S   P   K   G
271 ACG GCT ACA TAT TTC TGT GCA TTA TAT GGT AAC TCC CCT AAG GGG

+1   F   A   Y   W   G   Q   G   T   L   V   T   V   S   A   G
316 TTT GCT TAC TGG GGC CAA GGG ACT CTG GTC ACT GTC TCT GCA GGT

```

*linker*

## VL

```

+1   G   G   G   S   G   G   R   A   S   G   G   G   G   S   D
361 GGA GGC GGT TCA GGT GGG CGC GCC TCT GGC GGT GGC GGA TCG GAT

+1   I   Q   M   T   Q   S   P   K   F   L   L   V   S   A   G
406 ATT CAG ATG ACA CAG TCT CCC AAA TTC CTG CTT GTA TCA GCA GGA

```

FIG. 14-1

U.S. Patent

Apr. 25, 2006

Sheet 22 of 61

US 7,033,590 B1

```
+1   D   R   V   T   I   T   C   K   A   S   Q   S   V   S   N
451 GAC AGG GTT ACC ATA ACC TGC AAG GCC AGT CAG AGT GTG AGT AAT

+1   D   V   A   W   Y   Q   Q   K   P   G   Q   S   P   K   L
496 GAT GTA GCT TGG TAC CAA CAG AAG CCG GGG CAG TCT CCT AAA CTA

+1   L   M   Y   Y   A   S   N   R   Y   T   G   V   P   D   R
541 CTG ATG TAC TAT GCA TCC AAT CGC TAC ACT GGA GTC CCT GAT CGC

+1   F   T   G   S   G   Y   G   T   D   F   T   F   T   I   S
586 TTC ACT GGC AGT GGA TAT GGG ACG GAT TTC ACT TTC ACC ATC AGC

+1   T   V   Q   A   E   D   L   A   V   Y   F   C   Q   Q   D
631 ACT GTG CAG GCT GAA GAC CTG GCA GTT TAT TTC TGT CAG CAG GAT

+1   Y   G   S   P   P   T   F   G   G   G   T   K   L   E   I
676 TAT GGC TCT CCT CCC ACG TTC GGA GGG GGC ACC AAG CTG GAA ATT

+1   K   R
721 AAA CGG
```

**FIG. 14-2**

U.S. Patent

Apr. 25, 2006

Sheet 23 of 61

US 7,033,590 B1

VH

+1	E	V	Q	L	V	E	S	G	G	G	L	V	K	P	G
1	GAA	GTG	CAG	CTG	GTG	GAG	TCT	GGG	GGA	GGC	CTA	GTG	AAG	CCT	GGA
+1	G	S	L	K	L	S	C	A	A	S	G	F	T	F	S
46	GGG	TCC	CTG	AAA	CTC	TCC	TGT	GCA	GCC	TCT	GGA	TTC	ACT	TTC	AGT
+1	T	Y	T	M	S	W	V	R	Q	T	P	E	K	R	L
91	ACC	TAT	ACC	ATG	TCT	TGG	GTT	CGC	CAG	ACT	CCG	GAG	AAG	AGG	CTG
+1	E	W	V	A	T	I	S	S	G	G	S	Y	T	Y	Y
136	GAG	TGG	GTC	GCA	ACC	ATT	AGT	AGT	GGT	GGT	AGT	TAC	ACC	TAC	TAT
+1	P	D	S	V	R	G	R	F	T	I	S	R	D	N	A
181	CCA	GAC	AGT	GTG	AGG	GGC	CGA	TTC	ACC	ATC	TCC	AGA	GAC	AAT	GCC
+1	K	N	T	L	Y	L	Q	M	S	S	L	K	S	E	D
226	AAG	AAC	ACC	CTG	TAC	CTG	CAA	ATG	AGC	AGT	CTG	AAG	TCT	GAG	GAC
+1	T	A	M	Y	Y	C	T	R	D	G	G	H	G	Y	G
271	ACA	GCC	ATG	TAT	TAC	TGT	ACA	AGA	<b>GAT</b>	<b>GGG</b>	<b>GGA</b>	<b>CAC</b>	<b>GGG</b>	<b>TAC</b>	<b>GGT</b>
+1	S	S	F	D	Y	W	G	Q	G	T	T	L	T	V	S
316	<b>AGT</b>	<b>AGC</b>	<b>TTT</b>	<b>GAC</b>	<b>TAC</b>	TGG	GGC	CAA	GGC	ACC	ACT	CTC	ACA	GTC	TCC
	<i>linker</i>														
+1	S	G	G	G	S	G	G	R	A	S	G	G	G	G	G
361	TCA	GGT	GGA	GGC	GGT	TCA	GGT	GGG	CGC	GCC	TCT	GGC	GGT	GGC	GGA
	VL														
+1	S	Q	I	V	L	T	Q	S	P	L	S	L	P	V	S
406	TCG	CAA	ATT	GTG	CTC	ACC	CAG	TCT	CCA	CTC	TCC	CTG	CCT	GTC	AGT

FIG. 15-1

U.S. Patent

Apr. 25, 2006

Sheet 24 of 61

US 7,033,590 B1

```

+1   L   G   D   Q   A   S   I   S   C   R   S   S   Q   S   I
451 CTT GGA GAT CAA GCC TCC ATC TCT TGC AGA TCT AGT CAG AGC ATT

+1   V   H   S   N   G   N   T   Y   L   E   W   Y   L   Q   K
496 GTA CAT AGT AAT GGA AAC ACC TAT TTA GAA TGG TAC CTG CAG AAA

+1   P   G   Q   S   P   K   L   L   I   Y   K   V   S   N   R
541 CCA GGC CAG TCT CCA AAG CTC CTG ATC TAC AAA GTT TCC AAC CGA

+1   F   S   G   V   P   D   K   F   S   G   S   G   S   G   T
586 TTT TCT GGG GTC CCA GAC AAA TTC AGT GGC AGT GGA TCA GGG ACA

+1   D   F   T   L   K   I   S   R   V   E   A   E   D   L   G
631 GAT TTC ACA CTC AAG ATC AGC AGA GTG GAG GCT GAG GAT CTG GGA

+1   V   Y   Y   C   F   Q   G   S   H   V   P   W   T   F   G
676 GTT TAT TAC TGC TTT CAA GGT TCA CAT GTT CCG TGG ACG TTC GGT

+1   G   G   T   K   L   E   I   K   R
721 GGA GGC ACC AAG CTG GAA ATC AAA CGG

```

**FIG. 15-2**

U.S. Patent

Apr. 25, 2006

Sheet 25 of 61

US 7,033,590 B1

```

+1 E V Q L Q E S G G G L V K P G
1  GAG GTG CAG CTT CAG GAG TCA GGG GGA GGC TTA GTG AAG CCT GGA

+1 G S L K L S C A A S G F T F S
46  GGG TCC CTG AAA CTC TCC TGT GCA GCC TCT GGA TTC ACT TTC AGT

+1 S Y T M S W V R Q T P E K R L
91  AGC TAT ACC ATG TCT TGG GTT CGC CAG ACT CCG GAG AAG AGG CTG

+1 E W V A T I S S G G S S T Y Y
136 GAG TGG GTC GCA ACC ATT AGT AGT GGT GGT AGT TCC ACC TAC TAT

+1 P D S V K G R F T I S R D N A
181 CCA GAC AGT GTG AAG GGC CGA TTC ACC ATC TCC AGA GAC AAT GCC

+1 K N T L Y L Q M S S L R S E D
226 AAG AAC ACC CTG TAC CTG CAA ATG AGC AGT CTG AGG TCT GAG GAC

+1 T A M Y Y C T R E G G G F T V
271 ACA GCC ATG TAT TAC TGT ACA AGA GAG GGG GGT GGT TTC ACC GTC

+1 N W Y F D V W G A G T L V T V
316 AAC TGG TAC TTC GAT GTC TGG GGC GCA GGG ACT CTG GTC ACT GTC

      linker
+1 S A G G G G S G G R A S G G G
361 TCT GCA GGT GGA GGC GGT TCA GGT GGG CGC GCC TCT GGC GGT GGC

      VL
+1 G S E N V L T Q S P A S L A V
406 GGA TCG GAA AAT GTG CTC ACC CAG TCT CCA GCT TCT TTG GCT GTG

```

FIG. 16-1

U.S. Patent

Apr. 25, 2006

Sheet 26 of 61

US 7,033,590 B1

```

+1 S L G Q R A T I S C R A S E S
451 TCT CTA GGG CAG AGG GCC ACC ATA TCC TGC AGA GCC AGT GAA AGT

+1 V D S Y G Y N F M H W Y Q Q I
496 GTT GAT AGT TAT GGC TAT AAT TTT ATG CAC TGG TAT CAG CAG ATA

+1 P G Q P P K L L I Y R A S N L
541 CCA GGA CAG CCA CCC AAA CTC CTC ATC TAT CGT GCA TCC AAC CTA

+1 E S G I P A R F S G S G S R T
586 GAG TCT GGG ATC CCT GCC AGG TTC AGT GGC AGT GGG TCT AGG ACA

+1 D F T L T I N P V E A D D V A
631 GAC TTC ACC CTC ACC ATT AAT CCT GTG GAG GCT GAT GAT GTT GCA

+1 T Y Y C Q Q S N E D P L T F G
676 ACC TAT TAC TGT CAG CAA AGT AAT GAG GAT CCG CTC ACG TTC GGT

+1 T G T R L E I K R
721 ACT GGG ACC AGA CTG GAA ATA AAA CGG

```

**FIG. 16-2**



+1 E V Q L Q E S G G L V K P G G S L K L  
 1 GAG GTG CAG CTT CAG GAG TCA GGG GGA GGC TTA GTG AAG CCT GGA GGG TCC CTG AAA CTC  
 CTC CAC GTC GAA GTC CTC AGT CCC CCT CCG AAT CAC TTC GGA CCT CCC AGG GAC TTT GAG  
  
 +1 S C A A S G G F I F S S Y T M S W V R Q T  
 61 TCC TGT GCA GCC TCT GGA TTC ATT TTT AGT AGT TAT ACC ATG TCT TGG GTT CGC CAG ACT  
 AGG ACA CGT CGG AGA CCT AAG TAA AAA TCA TCA ATA TGG TAC AGA ACC CAA GCG GTC TGA  
  
 +1 P E K R L E W V A T I S S G G S S T Y Y  
 121 CCG GAG AAG AGG CTG GAG TGG GTC GCA ACC ATT AGT AGT AGT GGT GGT AGT TCC ACC TAC TAT  
 GGC CTC TTC TCC GAC CTC ACC CAG CGT TGG TAA TCA TCA CCA CCA TCA AGG TGG ATG ATA  
  
 +1 P D S V K G R F T I S R D N A K N T L Y  
 181 CCA GAC AGT GTG AAG GGC CGA TTC ACC ATC TCC AGA GAC AAT GCC AAG AAC ACC CTG TAC  
 GGT CTG TCA CAC TTC CCG GCT AAG TGG TAG AGG TCT CTG TTA CGG TTC TTG TGG GAC ATG  
  
 +1 L Q M S S L K S E D T A M Y H C T R E G  
 241 CTG CAA ATG AGC AGT CTG AAG TCT GAG GAC ACA GCC ATG TAT CAC TGT ACA AGA GAG GGG  
 GAC GTT TAC TCG TCA GAC TTC AGA CTC CTG TGT CGG TAC ATA GTG ACA TGT TCT CTC CCC  
  
 +1 G G Y Y V N W Y F D V W G A G T L T V  
 301 GGT GGT TAT TAC GTC AAC TGG TAC TTC GAT GTC TGG GGC GCA GGC ACC ACT CTC ACA GTC  
 CCA CCA ATA ATG CAG TTG ACC ATG AAG CTA CAG ACC CCG CGT CCG TGG TGA GAG TGT CAG  
  
 +1 S S G G G S G G R A S G G G G S D I E  
 361 TCC TCA GGT GGA GGC GGT TCA GGT GGC CGC GCC TCT GGC GGT GGC GGA TCG GAC ATT GAG  
 AGG AGT CCA CCT CCG CCA AGT CCA CCC GCG CGG AGA CCG CCA CCG CCT AGC CTG TAA CTC  
  
 +1 L T Q S P A S L A V S L G Q R A T I S C  
 421 CTC ACN CAG TCT CCA GCT TCT TTG GCT GTG TCT CTA GGC CAG AGG GCC ACC ATA TCC TGC  
 GAG TGN GTC AGA GGT CGA AGA AAC CGA CAC AGA GAT CCC GTC TCC CGG TGG TAT AGG ACG

*linker**VL***FIG. 17-1**

```

+1  R  A  S  E  S  V  D  S  Y  G  K  S  F  M  H  W  Y  Q  Q  K
481  AGA GCC AGT GAA AGT GTT GAT AGT TAT GGC AAG AGT TTT ATG CAC TGG TAC CAG CAG AAA
    TCT CGG TCA CTT TCA CAA CTA TCA ATA CCG TTC TCA AAA TAC GTG ACC ATG GTC GTC TTT

+1  P  G  Q  P  P  K  L  L  I  Y  R  A  S  N  L  E  S  G  I  P
541  CCA GGG CAG CCA CCC AAA CTC CTC ATC TAT CGT GCA TCC AAC CTA GAA TCT GGG ATC CCT
    GGT CCC GTC GGT GGG TTT GAG GAG TAG ATA GCA CGT AGG TTG GAT CTT AGA CCC TAG GGA

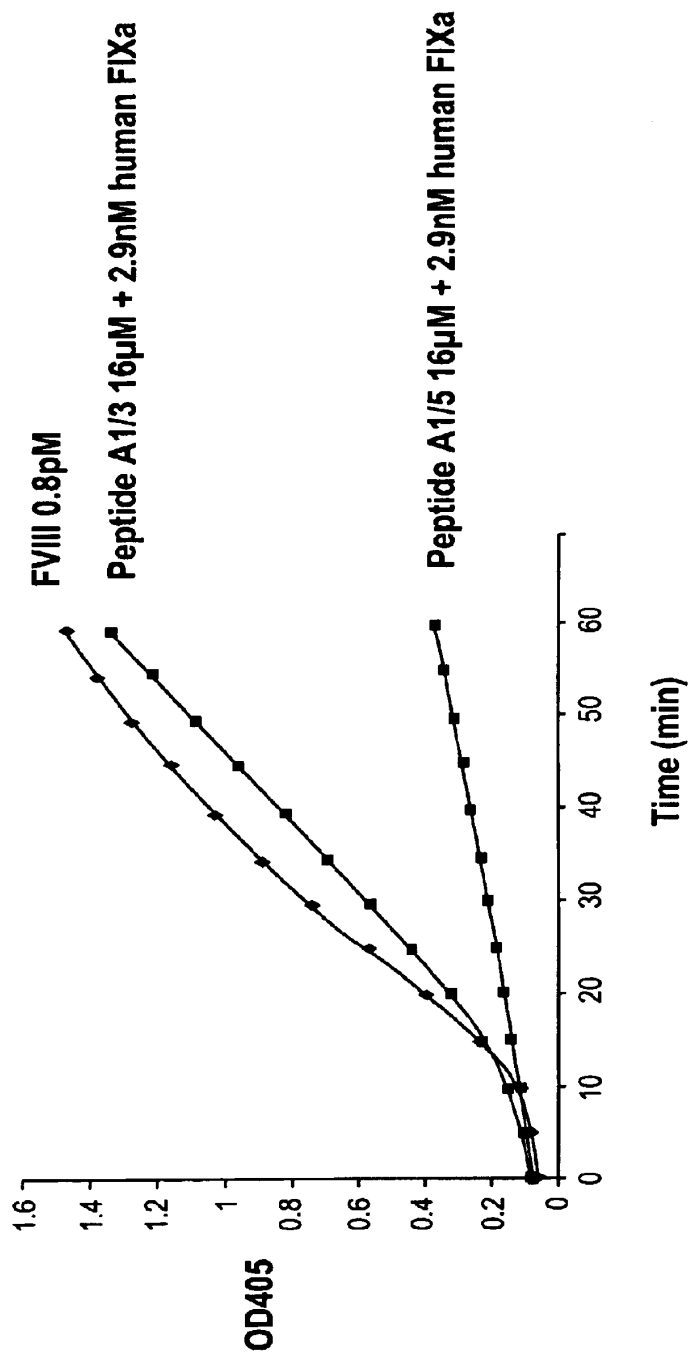
+1  A  R  F  S  G  S  G  S  R  T  D  F  T  L  T  I  N  P  V  E
601  GCC AGG TTC AGT GGC AGT GGG TCT AGG ACA GAC TTC ACC CTC ACC ATT AAT CCT GTG GAG
    CGG TCC AAG TCA CCG TCA CCC AGA TCC TGT CTG AAG TGG GAG TGG TAA TTA GGA CAC CTC

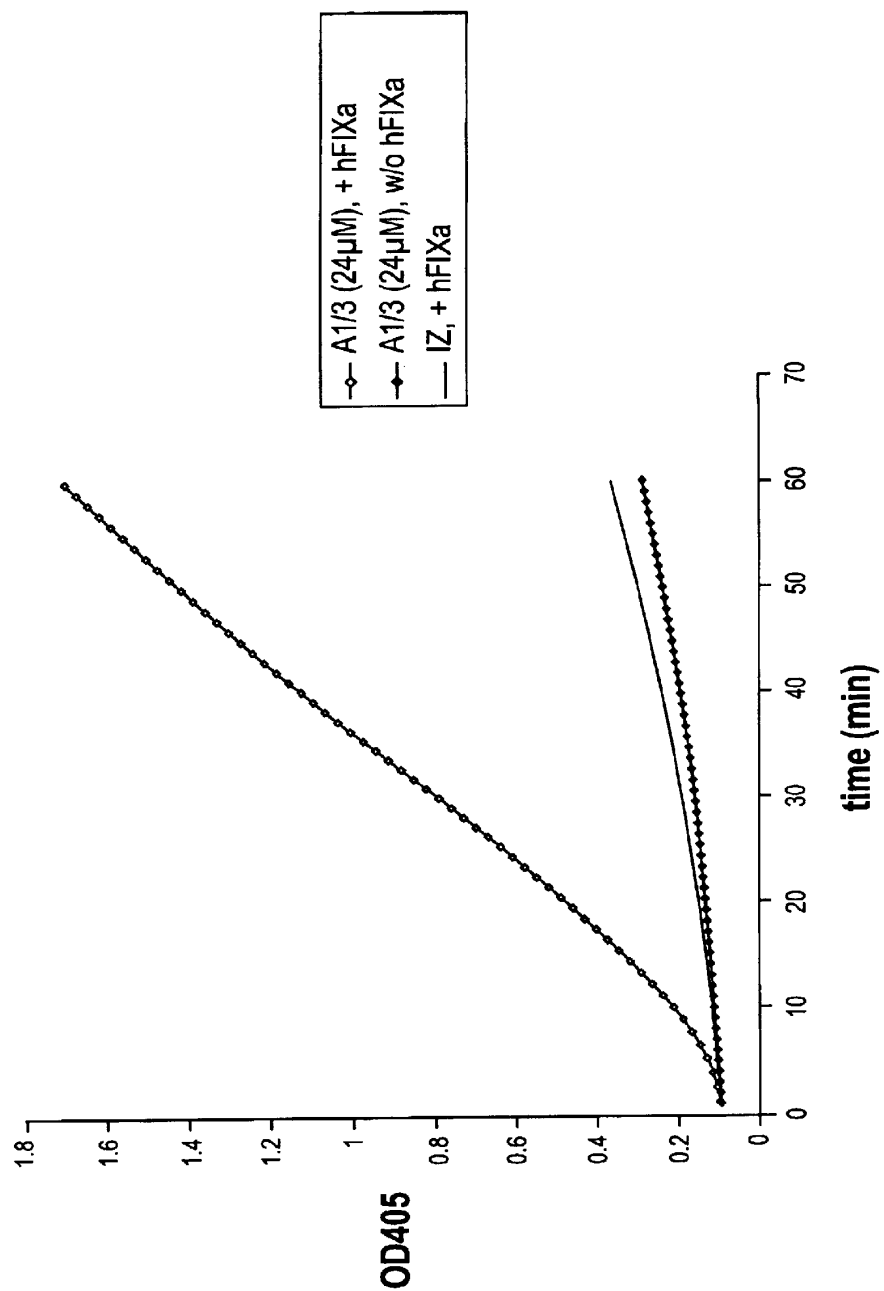
+1  A  D  D  V  A  T  Y  Y  C  Q  Q  S  N  E  D  P  L  T  F  G
661  GCT GAT GAT GTT GCN ACC TAT TAC TGT CAG CAA AGT AAT GAG GAT CCC CTC ACG TTC GGT
    CGA CTA CTA CAA CGN TGG ATA ATG ACA GTC GTT TCA TTA CTC CTA GGG GAG TGC AAG CCA

+1  A  G  T  R  L  E  I  K  R
721  GCT GGG ACC AGA CTG GAA ATA AAA CGG
    CGA CCC TGG TCT GAC CTT TAT TTT GCC

```

FIG. 17-2

**FIG. 18**

**FIG. 19**

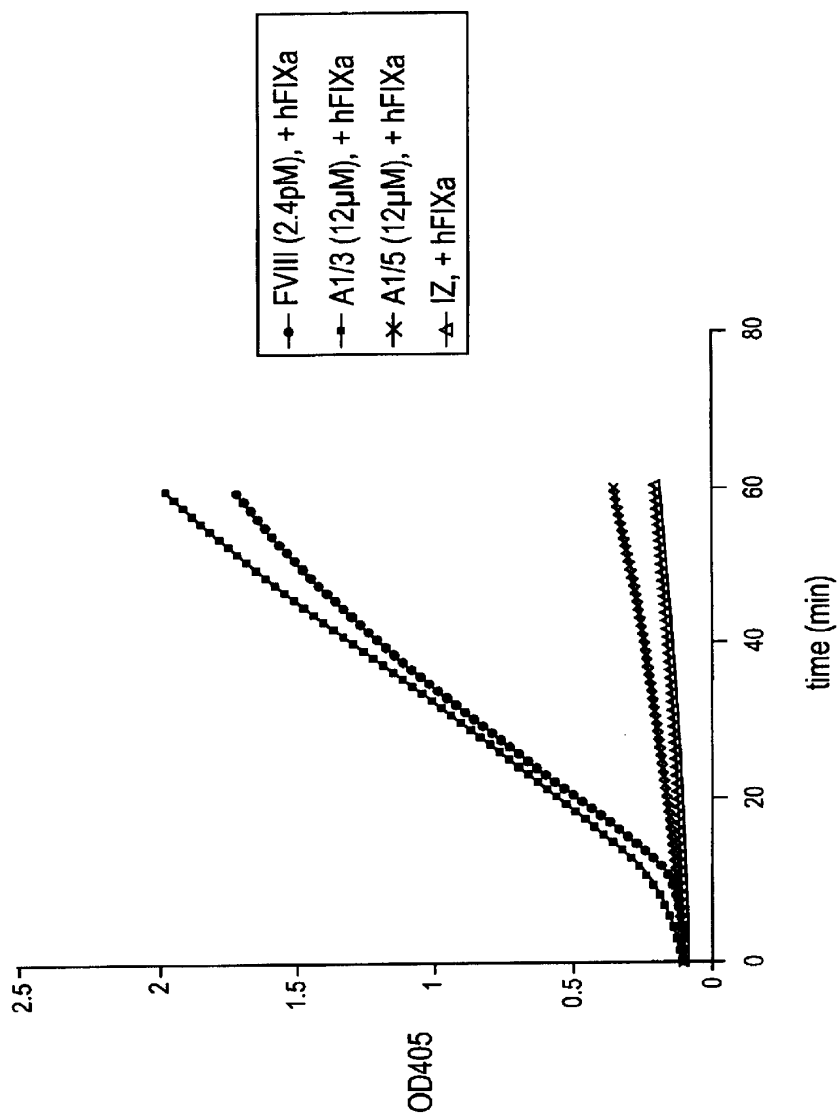
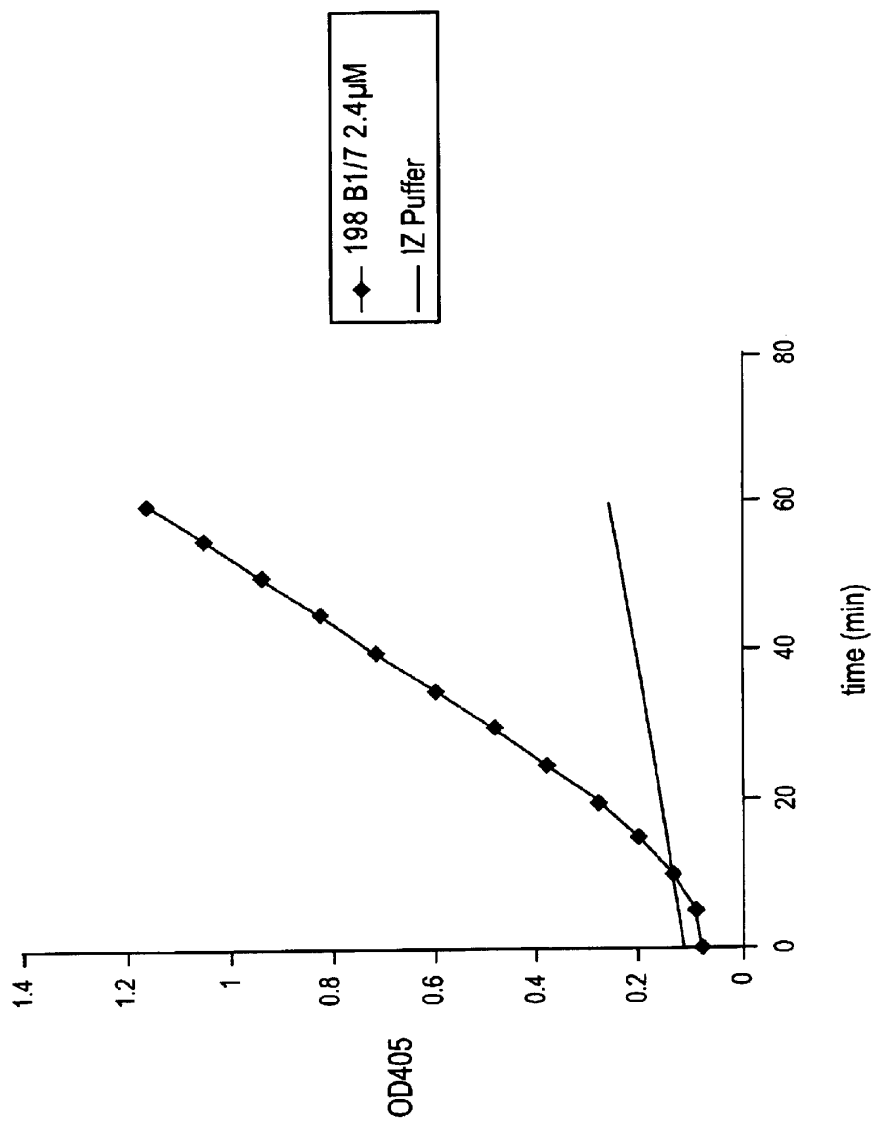
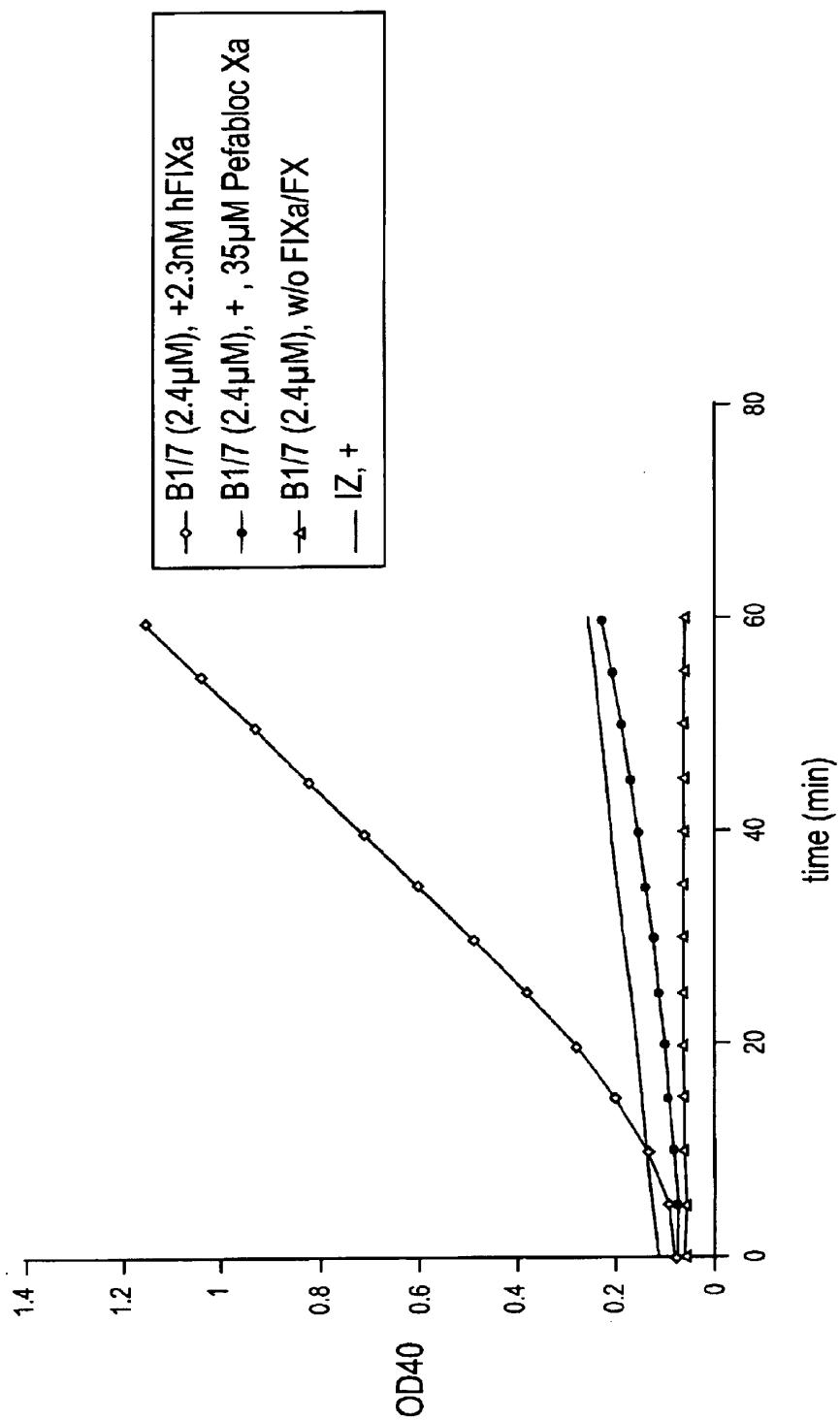


FIG. 20

**FIG. 21**

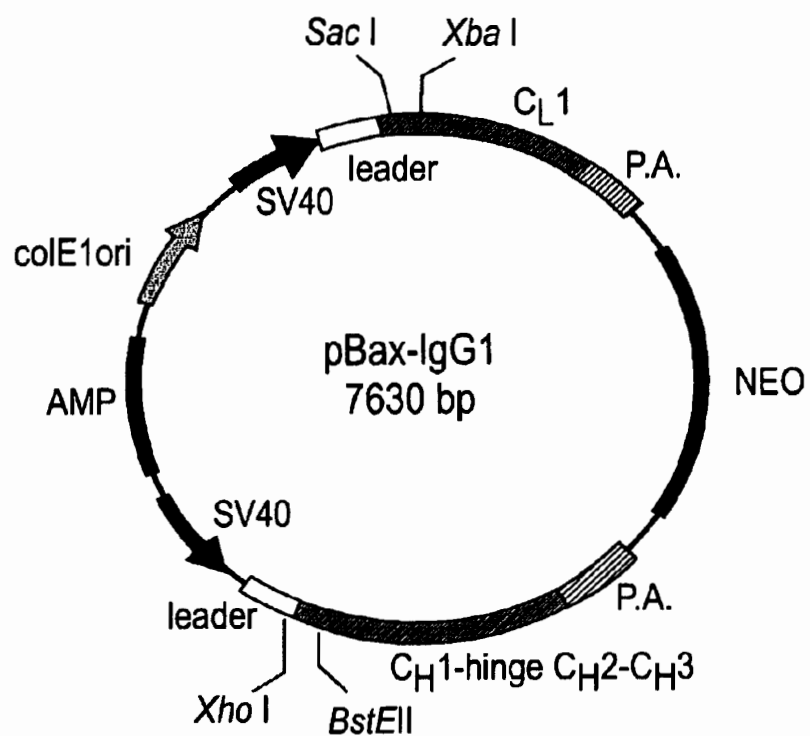
**FIG. 22**

U.S. Patent

Apr. 25, 2006

Sheet 34 of 61

US 7,033,590 B1



**FIG. 23**



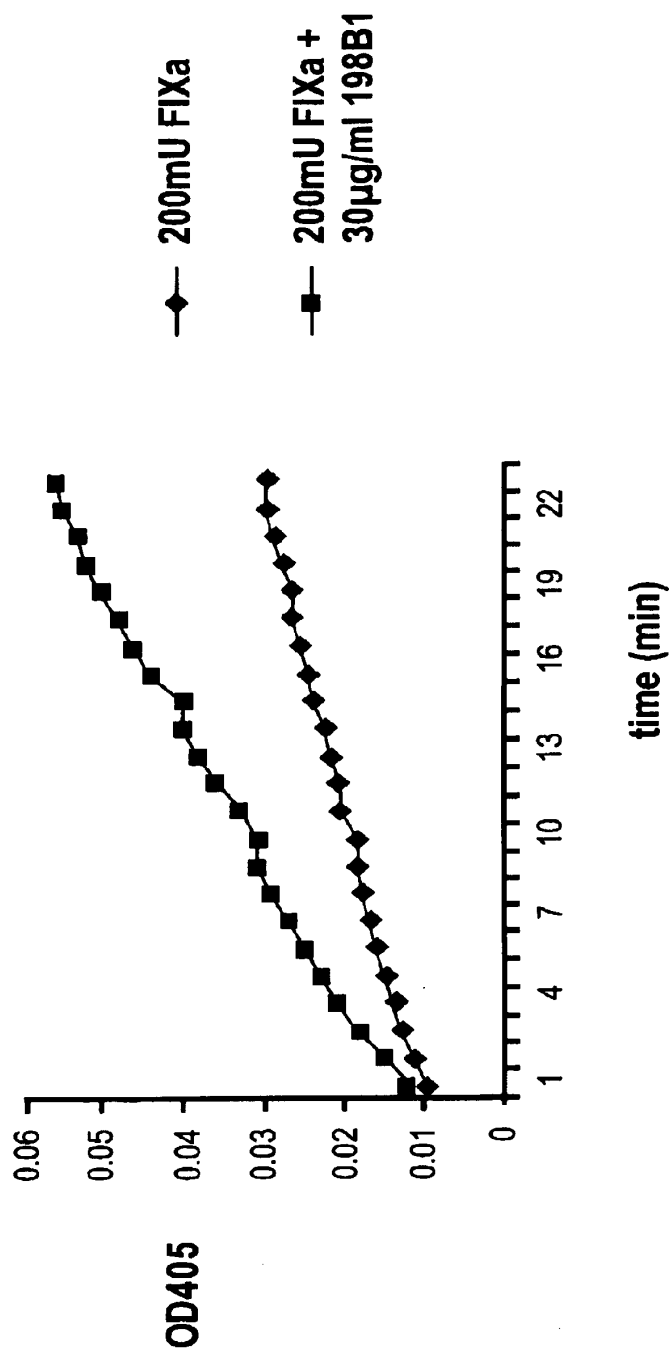
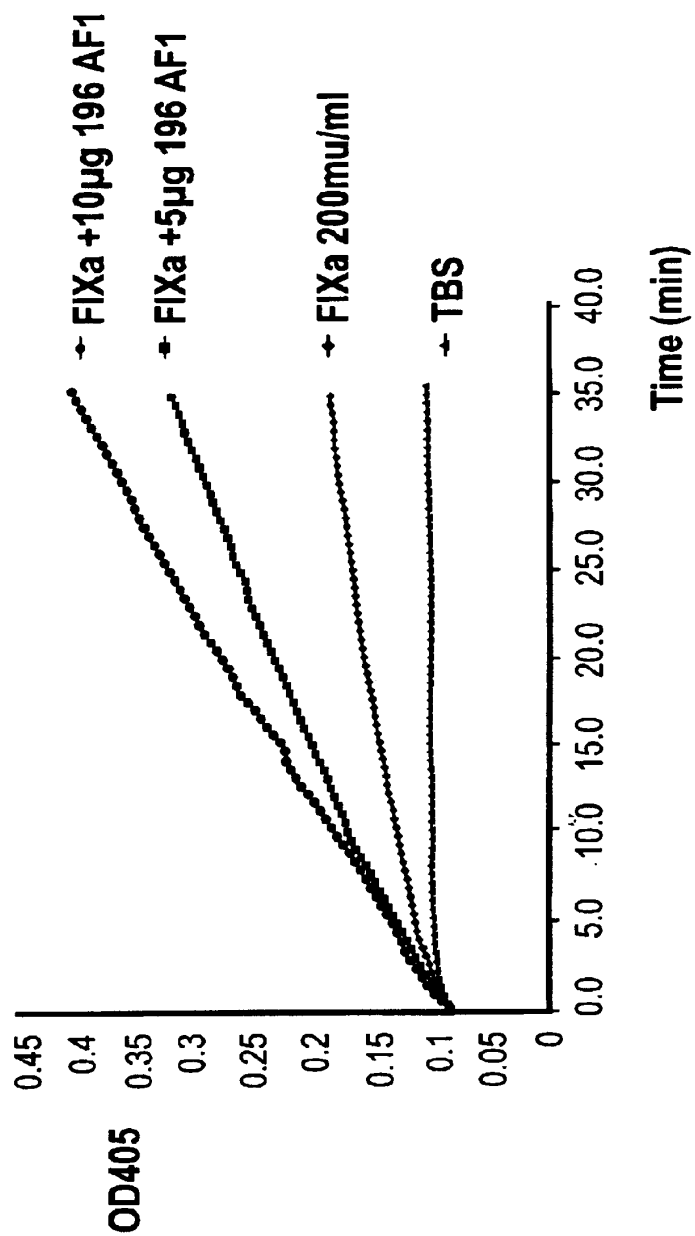


FIG. 24A

**FIG. 24B**

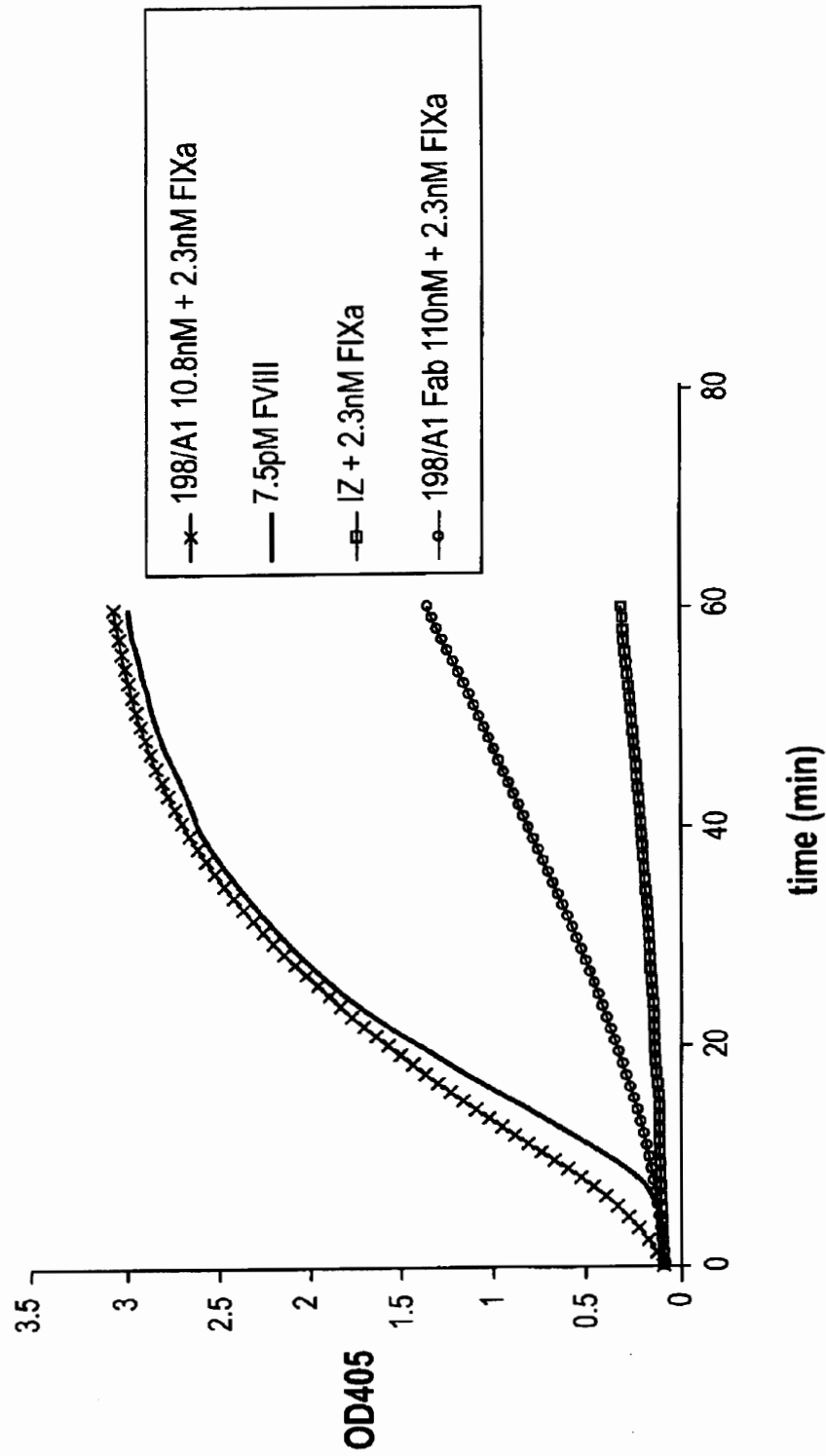


FIG. 25

U.S. Patent

Apr. 25, 2006

Sheet 38 of 61

US 7,033,590 B1

PelB-Leader

```

+1  M  K  Y  L  L  P  T  A  A  A  G  L  L  L
1   ATG AAA TAC CTA TTG CCT ACG GCA GCC GCT GGA TTG TTA TTA
    TAC TTT ATG GAT AAC GGA TGC CGT CGG CGA CCT AAC AAT AAT

+1  L  A  A  Q  P  A  M  A  |  E  V  K  L  V  E
43  CTC GCG GCC CAG CCG GCC ATG GCG | GAG GTG AAG CTG GTG GAG
    GAG CGC CGG GTC GGC CGG TAC CGC | CTC CAC TTC GAC CAC CTC

+1  S  G  G  G  L  V  K  P  G  G  S  L  K  L
85  TCT GGG GGA GGC TTA GTG AAG CCT GGA GGG TCC CTG AAA CTC
    AGA CCC CCT CCG AAT CAC TTC GGA CCT CCC AGG GAC TTT GAG

+1  S  C  A  A  S  G  F  T  F  S  S  Y  T  M
127 TCC TGT GCA GCC TCT GGA TTC ACT TTC AGT AGC TAT ACC ATG
    AGG ACA CGT CGG AGA CCT AAG TGA AAG TCA TCG ATA TGG TAC

+1  S  W  V  R  Q  T  P  E  K  R  L  E  W  V
169 TCT TGG GTT CGC CAG ACT CCG GAG AAG AGG CTG GAG TGG GTC
    AGA ACC CAA GCG GTC TGA GGC CTC TTC TCC GAC CTC ACC CAG

+1  A  T  I  S  S  G  G  S  S  T  Y  Y  P  D
211 GCA ACC ATT AGT AGT GGN GGT AGT TCC ACC TAC TAT CCA GAC
    CGT TGG TAA TCA TCA CCN CCA TCA AGG TGG ATG ATA GGT CTG

+1  S  V  K  G  R  F  T  I  S  R  D  N  A  K
253 AGT GTG AAG GGC CGA TTC ACC ATC TCC AGA GAC AAT GCC AAG
    TCA CAC TTC CCG GCT AAG TGG TAG AGG TCT CTG TTA CGG TTC

+1  N  T  L  Y  L  Q  M  S  S  L  R  S  E  D
295 AAC ACC CTG TAC CTG CAA ATG AGC AGT CTG AGG TCT GAG GAC
    TTG TGG GAC ATG GAC GTT TAC TCG TCA GAC TCC AGA CTC CTG

+1  T  A  M  Y  Y  C  T  R  E  G  G  G  F  T
337 ACA GCC ATG TAT TAC TGT ACA AGA GAG GGG GGT GGT TTC ACC
    TGT CGG TAC ATA ATG ACA TGT TCT CTC CCC CCA CCA AAG TGG

+1  V  N  W  Y  F  D  V  W  G  A  G  T  S  V
379 GTC AAC TGG TAC TTC GAT GTC TGG GGC GCA GGA ACC TCA GTC
    CAG TTG ACC ATG AAG CTA CAG ACC CCG CGT CCT TGG AGT CAG

+1  T  V  S  S  |  G  G  G  G  S  G  G  R  A  S
421 ACC GTC TCC TCA | GGT GGA GGC GGT TCA GGT GGG CGC GCC TCT
    TGG CAG AGG AGT | CCA CCT CCG CCA AGT CCA CCC GCG CGG AGA

```

linker

FIG. 26-1

## US 7,033,590 B1

**FIG. 26-2**

U.S. Patent

Apr. 25, 2006

Sheet 40 of 61

US 7,033,590 B1

```

+1 L L I G D G M G D S E I T A
463 TTG CTG ATT GGC GAT GGG ATG GGG GAC TCG GAA ATT ACT GCC
AAC GAC TAA CCG CTA CCC TAC CCC CTG AGC CTT TAA TGA CCG

+1 A R N Y A E G A G G F F K G
505 GCA CGT AAT TAT GCC GAA GGT GCG GGC GGC TTT TTT AAA GGT
CGT GCA TTA ATA CGG CTT CCA CGC CCG CCG AAA AAA TTT CCA

+1 I D A L P L T G Q Y T H Y A
1051 ATA GAT GCC TTA CCG CTT ACC GGG CAA TAC ACT CAC TAT GCG
TAT CTA CGG AAT GGC GAA TGG CCC GTT ATG TGA GTG ATA CGC

+1 L N K K T G K P D Y V T D S
1093 CTG AAT AAA AAA ACC GGC AAA CCG GAC TAC GTC ACC GAC TCG
GAC TTA TTT TTT TGG CCG TTT GGC CTG ATG CAG TGG CTG AGC

+1 A A S A T A W S T G V K T Y
1135 GCT GCA TCA GCA ACC GCC TGG TCA ACC GGT GTC AAA ACC TAT
CGA CGT AGT CGT TGG CGG ACC AGT TGG CCA CAG TTT TGG ATA

+1 N G A L G V D I H E K D H P
1177 AAC GGC GCG CTG GGC GTC GAT ATT CAC GAA AAA GAT CAC CCA
TTG CCG CGC GAC CCG CAG CTA TAA GTG CTT TTT CTA GTG GGT

+1 T I L E M A K A A G L A T G
1219 ACG ATT CTG GAA ATG GCA AAA GCC GCA GGT CTG GCG ACC GGT
TGC TAA GAC CTT TAC CGT TTT CGG CGT CCA GAC CGC TGG CCA

+1 N V S T A E L Q D A T P A A
1261 AAC GTT TCT ACC GCA GAG TTG CAG GAT GCC ACG CCC GCT GCG
TTG CAA AGA TGG CGT CTC AAC GTC CTA CGG TGC GGG CGA CGC

+1 L V A H V T S R K C Y G P S
1303 CTG GTG GCA CAT GTG ACC TCG CGC AAA TGC TAC GGT CCG AGC
GAC CAC CGT GTA CAC TGG AGC GCG TTT ACG ATG CCA GGC TCG

+1 A T S E K C P G N A L E K G
1345 GCG ACC AGT GAA AAA TGT CCG GGT AAC GCT CTG GAA AAA GGC
CGC TGG TCA CTT TTT ACA GGC CCA TTG CGA GAC CTT TTT CCG

+1 G K G S I T E Q L L N A R A
1387 GGA AAA GGA TCG ATT ACC GAA CAG CTG CTT AAC GCT CGT GCC
CCT TTT CCT AGC TAA TGG CTT GTC GAC GAA TTG CGA GCA CCG

+1 D V T L G G G A K T F A E T
1429 GAC GTT ACG CTT GGC GGC GGC GCA AAA ACC TTT GCT GAA ACG
CTG CAA TGC GAA CCG CCG CCG CGT TTT TGG AAA CGA CTT TGC

```

FIG. 26-3

U.S. Patent

Apr. 25, 2006

Sheet 41 of 61

US 7,033,590 B1

```

+1 A T A G E W Q G K T L R E Q
1471 GCA ACC GCT GGT GAA TGG CAG GGA AAA ACG CTG CGT GAA CAG
      CGT TGG CGA CCA CTT ACC GTC CCT TTT TGC GAC GCA CTT GTC

+1 A Q A R G Y Q L V S D A A S
1513 GCA CAG GCG CGT GGT TAT CAG TTG GTG AGC GAT GCT GCC TCA
      CGT GTC CGC GCA CCA ATA GTC AAC CAC TCG CTA CGA CGG AGT

+1 L N S V T E A N Q Q K P L L
1555 CTG AAT TCG GTG ACG GAA GCG AAT CAG CAA AAA CCC CTG CTT
      GAC TTA AGC CAC TGC CTT CGC TTA GTC GTT TTT GGG GAC GAA

+1 G L F A D G N M P V R W L G
1177 GGC CTG TTT GCT GAC GGC AAT ATG CCA GTG CGC TGG CTA GGA
      CCG GAC AAA CGA CTG CCG TTA TAC GGT CAC GCG ACC GAT CCT

+1 P K A T Y H G N I D K P A V
1639 CCG AAA GCA ACG TAC CAT GGC AAT ATC GAT AAG CCC GCA GTC
      GGC TTT CGT TGC ATG GTA CCG TTA TAG CTA TTC GGG CGT CAG

+1 T C T P N P Q R N D S V P T
1681 ACC TGT ACG CCA AAT CCG CAA CGT AAT GAC AGT GTA CCA ACC
      TGG ACA TGC GGT TTA GGC GTT GCA TTA CTG TCA CAT GGT TGG

+1 L A Q M T D K A I E L L S K
1723 CTG GCG CAG ATG ACC GAC AAA GCC ATT GAA TTG TTG AGT AAA
      GAC CGC GTC TAC TGG CTG TTT CGG TAA CTT AAC AAC TCA TTT

+1 N E K G F F L Q V E G A S I
1765 AAT GAG AAA GGC TTT TTC CTG CAA GTT GAA GGT GCG TCA ATC
      TTA CTC TTT CCG AAA AAG GAC GTT CAA CTT CCA CGC AGT TAG

+1 D K Q D H A A N P C G Q I G
1807 GAT AAA CAG GAT CAT GCT GCG AAT CCT TGT GGG CAA ATT GGC
      CTA TTT GTC CTA GTA CGA CGC TTA GGA ACA CCC GTT TAA CCG

+1 E T V D L D E A V Q R A L E
1849 GAG ACG GTC GAT CTC GAT GAA GCC GTA CAA CGG GCG CTG GAA
      CTC TGC CAG CTA GAG CTA CTT CGG CAT GTT GCC CGC GAC CTT

+1 F A K K E G N T L V I V T A
1891 TTC GCT AAA AAG GAG GGT AAC ACG CTG GTC ATA GTC ACC GCT
      AAG CGA TTT TTC CTC CCA TTG TGC GAC CAG TAT CAG TGG CGA

+1 D H A H A S Q I V A P D T K
1933 GAT CAC GCC CAC GCC AGC CAG ATT GTT GCG CCG GAT ACC AAA
      CTA GTG CGG GTG CGG TCG GTC TAA CAA CGC GGC CTA TGG TTT

+1 A P G L T Q A L N T K D G A
1975 GCT CCG GGC CTC ACC CAG GCG CTA AAT ACC AAA GAT GGC GCA
      CGA GGC CCG GAG TGG GTC CGC GAT TTA TGG TTT CTA CCG CGT

```

FIG. 26-4

U.S. Patent

Apr. 25, 2006

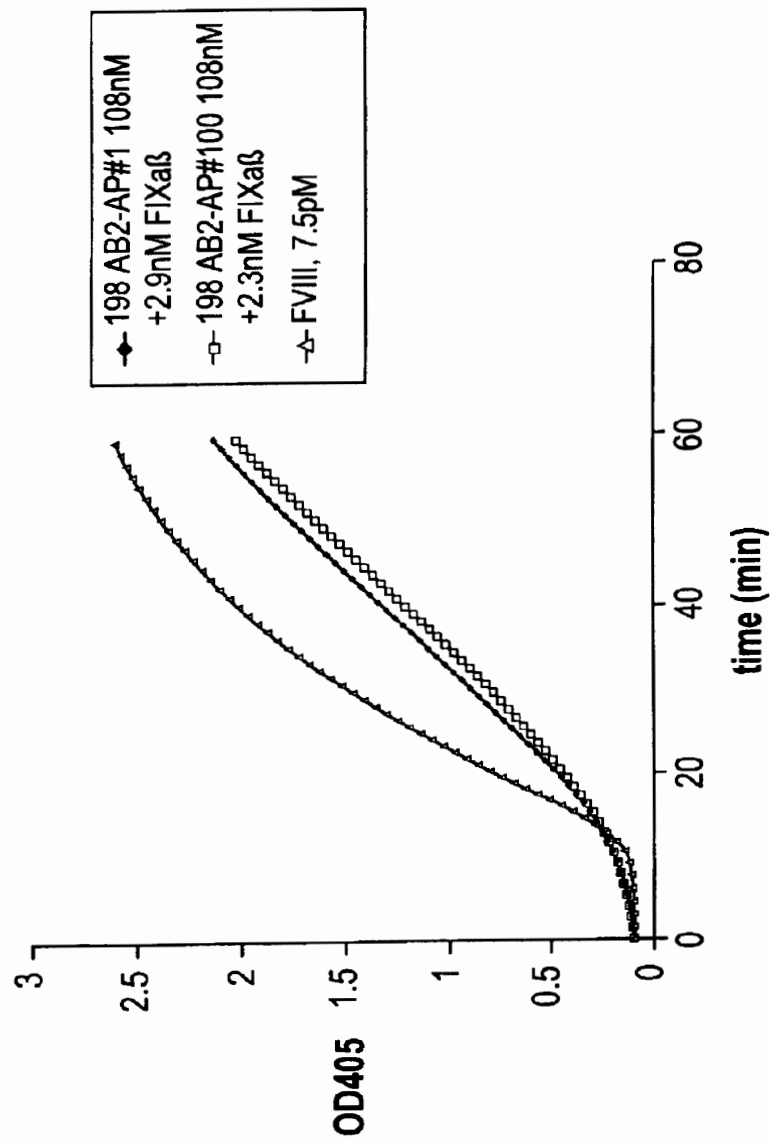
Sheet 42 of 61

US 7,033,590 B1

	+1	V	M	V	M	S	Y	G	N	S	E	E	D	S	Q
2017		GTG	ATG	GTG	ATG	AGT	TAC	GGG	AAC	TCC	GAA	GAG	GAT	TCA	CAA
		CAC	TAC	CAC	TAC	TCA	ATG	CCC	TTG	AGG	CTT	CTC	CTA	AGT	GTT
	+1	E	H	T	G	S	Q	L	R	I	A	A	Y	G	P
2059		GAA	CAT	ACC	GGC	AGT	CAG	TTG	CGT	ATT	GCG	GCG	TAT	GGC	CCG
		CTT	GTA	TGG	CCG	TCA	GTC	AAC	GCA	TAA	CGC	CGC	ATA	CCG	GGC
	+1	H	A	A	N	V	V	G	L	T	D	Q	T	D	L
2101		CAT	GCC	GCC	AAT	GTT	GTT	GGA	CTG	ACC	GAC	CAG	ACC	GAT	CTC
		GTA	CGG	CGG	TTA	CAA	CAA	CCT	GAC	TGG	CTG	GTC	TGG	CTA	GAG
	+1	F	Y	T	M	K	A	A	L	G	D	I	His tag		
2143		TTC	TAC	ACC	ATG	AAA	GCC	GCT	CTG	GGG	GAT	ATC	A	H	H
		AAG	ATG	TGG	TAC	TTT	CGG	CGA	GAC	CCC	CTA	TAG	GCA	CAC	CAT
													CGT	GTG	GTA
	+1	H	H	H	H	*									
2185		CAC	CAT	CAC	CAT	TAA									
		GTG	GTA	GTG	GTA	ATT									

FIG. 26-5



**FIG. 27**

U.S. Patent

Apr. 25, 2006

Sheet 44 of 61

US 7,033,590 B1

## PelB-Leader

```

+1  M   K   Y   L   L   P   T   A   A   A   G   L   L   L   L
1   ATG AAA TAC CTA TTG CCT ACG GCA GCC GCT GGA TTG TTA TTA CTC
    TAC TTT ATG GAT AAC GGA TGC CGT CGG CGA CCT AAC AAT AAT GAG

+1  A   A   Q   P   A   M   A   E   V   K   L   V   E   S   G
46  GCG GCC CAG CCG GCC ATG GCG GAG GTG AAG CTG GTG GAG TCT GGG
    CGC CGG GTC GGC CGG TAC CGC CTC CAC TTC GAC CAC CTC AGA CCC

+1  G   G   L   V   K   P   G   G   S   L   K   L   S   C   A
91  GGA GGC TTA GTG AAG CCT GGA GGG TCC CTG AAA CTC TCC TGT GCA
    CCT CCG AAT CAC TTC GGA CCT CCC AGG GAC TTT GAG AGG ACA CGT

+1  A   S   G   F   T   F   S   S   Y   T   M   S   W   V   R
136 GCC TCT GGA TTC ACT TTC AGT AGC TAT ACC ATG TCT TGG GTT CGC
    CGG AGA CCT AAG TGA AAG TCA TCG ATA TGG TAC AGA ACC CAA GCG

+1  Q   T   P   E   K   R   L   E   W   V   A   T   I   S   S
181 CAG ACT CCG GAG AAG AGG CTG GAG TGG GTC GCA ACC ATT AGT AGT
    GTC TGA GGC CTC TTC TCC GAC CTC ACC CAG CGT TGG TAA TCA TCA

+1  G   G   S   S   T   Y   Y   P   D   S   V   K   G   R   F
226 GGN GGT AGT TCC ACC TAC TAT CCA GAC AGT GTG AAG GGC CGA TTC
    CCN CCA TCA AGG TGG ATG ATA GGT CTG TCA CAC TTC CCG GCT AAG

+1  T   I   S   R   D   N   A   K   N   T   L   Y   L   Q   M
271 ACC ATC TCC AGA GAC AAT GCC AAG AAC ACC CTG TAC CTG CAA ATG
    TGG TAG AGG TCT CTG TTA CGG TTC TTG TGG GAC ATG GAC GTT TAC

+1  S   S   L   R   S   E   D   T   A   M   Y   Y   C   T   R
316 AGC AGT CTG AGG TCT GAG GAC ACA GCC ATG TAT TAC TGT ACA AGA
    TCG TCA GAC TCC AGA CTC CTG TGT CGG TAC ATA ATG ACA TGT TCT

+1  E   G   G   G   F   T   V   N   W   Y   F   D   V   W   G
361 GAG GGG GGT GGT TTC ACC GTC AAC TGG TAC TTC GAT GTC TGG GGC
    CTC CCC CCA CCA AAG TGG CAG TTG ACC ATG AAG CTA CAG ACC CCG

```

## Linker

```

+1  A   G   T   S   V   T   V   S   S   G   G   G   G   S   G
406 GCA GGA ACC TCA GTC ACC GTC TCC TCA GGT GGA GGC GGT TCA GGT
    CGT CCT TGG AGT CAG TGG CAG AGG AGT CCA CCT CCG CCA AGT CCA

```

## VL

```

+1  G   R   A   S   G   G   G   G   S   D   I   V   L   T   Q
451 GGG CGC GCC TCT GGC GGT GGC GGA TCG GAC ATT GTG CTG ACA CAG
    CCC GCG CGG AGA CCG CCA CCG CCT AGC CTG TAA CAC GAC TGT GTC

```

FIG. 28-1

U.S. Patent

Apr. 25, 2006

Sheet 45 of 61

US 7,033,590 B1

```

+1 X P A S L A V S L G Q R A T I
496 TNT CCA GCT TCT TTG GCT GTG TCT CTA GGG CAG AGG GCC ACC ATA
    ANA GGT CGA AGA AAC CGA CAC AGA GAT CCC GTC TCC CGG TGG TAT

+1 S C R A S E S V D S Y G Y N F
541 TCN TGC AGA GCC AGT GAA AGT GTT GAT AGT TAT GGC TAT AAT TTT
    AGN ACG TCT CGG TCA CTT TCA CAA CTA TCA ATA CCG ATA TTA AAA

+1 M H W Y Q Q I P G Q P P K L L
586 ATG CAC TGG TAT CAG CAG ATA CCA GGA CAG CCA CCC AAA CTC CTC
    TAC GTG ACC ATA GTC GTC TAT GGT CCT GTC GGT GGG TTT GAG GAG

+1 I Y R A S N L E S G I P A R F
631 ATC TAT CGT GCA TCC AAC CTA GAG TCT GGG ATC CCT GCC AGG TTC
    TAG ATA GCA CGT AGG TTG GAT CTC AGA CCC TAG GGA CGG TCC AAG

+1 S G S G S R T D F T L T I N P
676 AGT GGC AGT GGG TCT AGG ACA GAC TTC ACC CTC ACC ATT AAT CCT
    TCA CCG TCA CCC AGA TCC TGT CTG AAG TGG GAG TGG TAA TTA GGA

+1 V E A D D V A T Y Y C Q Q S N
721 GTG GAG GCT GAT GAT GTT GCA ACC TAT TAC TGT CAG CAA AGT AAT
    CAC CTC CGA CTA CTA CAA CGT TGG ATA ATG ACA GTC GTT TCA TTA

+1 E D P L T F G T G T R L E I K
766 GAG GAT CCG CTC ACG TTC GGT ACT GGG ACC AGA CTG GAA ATA AAA
    CTC CTA GGC GAG TGC AAG CCA TGA CCC TGG TCT GAC CTT TAT TTT

          Spacer      Hinge                      Helix

+1 R A A A P K P S T P P G S S R
811 CGG GCG GCC GCA CCG AAG CCT TCC ACT CCG CCC GGG TCT TCC CGT
    GCC CGC CGG CGT GGC TTC GGA AGG TGA GGC GGG CCC AGA AGG GCA

+1 M K Q L E D K V E E L L S K N
856 ATG AAA CAG CTG GAA GAC AAA GTA GAG GAG CTC CTT AGC AAG AAC
    TAC TTT GTC GAC CTT CTG TTT CAT CTC CTC GAG GAA TCG TTC TTG

+1 Y H L E N E V A R L K K L V G
901 TAC CAT CTA GAA AAC GAG GTA GCT CGT CTG AAA AAG CTT GTT GGT
    ATG GTA GAT CTT TTG CTC CAT CGA GCA GAC TTT TTC GAA CAA CCA

          Spacer      His-tag
+1 E R G G H H H H H *
946 GAA CGT GGT GGT CAC CAT CAC CAT CAC CAT TAA
    CTT GCA CCA CCA GTG GTA GTG GTA GTG GTA ATT

```

FIG. 28-2

U.S. Patent

Apr. 25, 2006

Sheet 46 of 61

US 7,033,590 B1

## PelB-Leader

```

+1  M  K  Y  L  L  P  T  A  A  A  G  L  L  L
1   ATG AAA TAC CTA TTG CCT ACG GCA GCC GCT GGA TTG TTA TTA
    TAC TTT ATG GAT AAC GGA TGC CGT CGG CGA CCT AAC AAT AAT

+1  L  A  A  Q  P  A  M  A  |  E  V  Q  L  Q  Q
43  CTC GCG GCC CAG CCG GCC ATG GCC | GAG GTT CAG CTT CAG CAG
    GAG CGC CGG GTC GGC CGG TAC CGG | CTC CAA GTC GAA GTC GTC

+1  S  G  P  E  L  V  K  P  G  A  S  V  K  I
85  TCT GGA CCT GAG CTG GTG AAG CCC GGG GCC TCA GTG AAG ATT
    AGA CCT GGA CTC GAC CAC TTC GGG CCC CGG AGT CAC TTC TAA

+1  S  C  K  A  S  G  Y  A  F  S  S  S  W  M
127 TCC TGC AAA GCT TCT GGC TAC GCA TTC AGT AGC TCT TGG ATG
    AGG ACG TTT CGA AGA CCG ATG CGT AAG TCA TCG AGA ACC TAC

+1  N  W  V  K  Q  R  P  G  Q  G  L  E  W  I
169 AAC TGG GTG AAG CAG AGG CCT GGA CAG GGT CTT GAG TGG ATT
    TTG ACC CAC TTC GTC TCC GGA CCT GTC CCA GAA CTC ACC TAA

+1  G  R  I  Y  P  G  N  G  D  T  N  Y  N  G
211 GGA CGG ATT TAT CCT GGA AAT GGA GAT ACT AAC TAC AAT GGG
    CCT GCC TAA ATA GGA CCT TTA CCT CTA TGA TTG ATG TTA CCC

+1  K  F  K  G  K  A  T  L  T  A  D  K  S  S
253 AAG TTC AAG GGC AAG GCC ACA CTG ACT GCA GAC AAA TCC TCC
    TTC AAG TTC CCG TTC CGG TGT GAC TGA CGT CTG TTT AGG AGG

+1  S  T  A  Y  M  Q  L  S  S  L  T  S  V  D
295 AGC ACA GCC TAC ATG CAG CTC AGC AGC CTG ACC TCT GTG GAC
    TCG TGT CGG ATG TAC GTC GAG TCG TCG GAC TGG AGA CAC CTG

+1  S  A  V  Y  F  C  A  D  G  N  V  Y  Y  Y
337 TCT GCG GTC TAT TTC TGT GCA GAT GGT AAC GTA TAT TAC TAT
    AGA CGC CAG ATA AAG ACA CGT CTA CCA TTG CAT ATA ATG ATA

+1  A  M  D  Y  W  G  Q  G  T  S  V  T  V  S
379 GCT ATG GAC TAC TGG GGT CAA GGA ACC TCA GTC ACC GTC TCC
    CGA TAC CTG ATG ACC CCA GTT CCT TGG AGT CAG TGG CAG AGG

```

## Linker

```

+1  S  |  G  G  G  G  S  G  G  R  A  S  G  G  G
421 TCA | GGT GGA GGC GGT TCA GGT GGG CGC GCC TCT GGC GGT GGC
    AGT | CCA CCT CCG CCA AGT CCA CCC GCG CGG AGA CCG CCA CCG

+1  G  S  |  Q  I  V  L  T  Q  S  P  A  S  L  A
463 GGA TCG | CAA ATT GTT CTC ACC CAG TCT CCT GCT TCC TTA GCT
    CCT AGC | GTT TAA CAA GAG TGG GTC AGA GGA CGA AGG AAT CGA

```

FIG. 29-1

U.S. Patent

Apr. 25, 2006

Sheet 47 of 61

US 7,033,590 B1

```

+1  V  S  L  G  Q  R  A  T  I  S  C  R  A  S
505  GTA TCT CTG GGG CAG AGG GCC ACC ATC TCA TGC AGG GCC AGC
    CAT AGA GAC CCC GTC TCC CGG TGG TAG AGT ACG TCC CGG TCG

+1  K  S  V  S  T  S  G  Y  S  Y  M  H  W  Y
547  AAA AGT GTC AGT ACA TCT GGC TAT AGT TAT ATG CAC TGG TAC
    TTT TCA CAG TCA TGT AGA CCG ATA TCA ATA TAC GTG ACC ATG

+1  Q  Q  K  P  G  Q  P  P  K  L  L  I  Y  L
589  CAA CAG AAA CCA GGA CAG CCA CCC AAA CTC CTC ATC TAT CTT
    GTT GTC TTT GGT CCT GTC GGT GGG TTT GAG GAG TAG ATA GAA

+1  A  S  N  L  E  S  G  V  P  A  R  F  S  G
631  GCA TCC AAC CTA GAA TCT GGG GTC CCT GCC AGG TTC AGT GGC
    CGT AGG TTG GAT CTT AGA CCC CAG GGA CGG TCC AAG TCA CCG

+1  S  G  S  G  T  D  F  T  L  N  I  H  P  V
679  AGT GGG TCT GGG ACA GAC TTC ACC CTC AAC ATC CAT CCT GTG
    TCA CCC AGA CCC TGT CTG AAG TGG GAG TTG TAG GTA GGA CAC

+1  E  E  E  D  A  A  T  Y  Y  C  Q  H  S  R
715  GAG GAG GAG GAT GCT GCA ACC TAT TAC TGT CAG CAC AGT AGG
    CTC CTC CTC CTA CGA CGT TGG ATA ATG ACA GTC GTG TCA TCC

+1  E  L  P  R  T  F  G  G  G  T  K  L  E  I
757  GAG CTT CCT CGG ACG TTC GGT GGA GGC ACC AAG CTG GAA ATC
    CTC GAA GGA GCC TGC AAG CCA CCT CCG TGG TTC GAC CTT TAG

+1  K  R  |  Spacer  |  Alkaline phosphatase
799  AAA CGG | GCG GCC GCA GCC | CGG GCA CCA GAA ATG CCT GTT CTG
    TTT GCC | CGC CGG CGT CGG | GCC CGT GGT CTT TAC GGA CAA GAC

+1  E  N  R  A  A  Q  G  D  I  T  A  P  G  G
841  GAA AAC CGG GCT GCT CAG GGC GAT ATT ACT GCA CCC GGC GGT
    CTT TTG GCC CGA CGA GTC CCG CTA TAA TGA CGT GGG CCG CCA

+1  A  R  R  L  T  G  D  Q  T  A  A  L  R  D
883  GCT CGC CGT TTA ACG GGT GAT CAG ACT GCC GCT CTG CGT GAT
    CGA GCG GCA AAT TGC CCA CTA GTC TGA CGG CGA GAC GCA CTA

+1  S  L  S  D  K  P  A  K  N  I  I  L  L  I
925  TCT CTT AGC GAT AAA CCT GCA AAA AAT ATT ATT TTG CTG ATT
    AGA GAA TCG CTA TTT GGA CGT TTT TTA TAA TAA AAC GAC TAA

+1  G  D  G  M  G  D  S  E  I  T  A  A  R  N
967  GGC GAT GGG ATG GGG GAC TCG GAA ATT ACT GCC GCA CGT AAT
    CCG CTA CCC TAC CCC CTG AGC CTT TAA TGA CGG CGT GCA TTA

```

**FIG. 29-2**

U.S. Patent

Apr. 25, 2006

Sheet 48 of 61

US 7,033,590 B1

```

+1   Y   A   E   G   A   G   G   F   F   K   G   I   D   A
1009 TAT GCC GAA GGT GCG GGC GGC TTT TTT AAA GGT ATA GAT GCC
     ATA CGG CTT CCA CGC CCG CCG AAA AAA TTT CCA TAT CTA CGG

+1   L   P   L   T   G   Q   Y   T   H   Y   A   L   N   K
1051 TTA CCG CTT ACC GGG CAA TAC ACT CAC TAT GCG CTG AAT AAA
     AAT GGC GAA TGG CCC GTT ATG TGA GTG ATA CGC GAC TTA TTT

+1   K   T   G   K   P   D   Y   V   T   D   S   A   A   S
1093 AAA ACC GGC AAA CCG GAC TAC GTC ACC GAC TCG GCT GCA TCA
     TTT TGG CCG TTT GGC CTG ATG CAG TGG CTG AGC CGA CGT AGT

+1   A   T   A   W   S   T   G   V   K   T   Y   N   G   A
1135 GCA ACC GCC TGG TCA ACC GGT GTC AAA ACC TAT AAC GGC GCG
     CGT TGG CCG ACC AGT TGG CCA CAG TTT TGG ATA TTG CCG CGC

+1   L   G   V   D   I   H   E   K   D   H   P   T   I   L
1177 CTG GGC GTC GAT ATT CAC GAA AAA GAT CAC CCA ACG ATT CTG
     GAC CCG CAG CTA TAA GTG CTT TTT CTA GTG GGT TGC TAA GAC

+1   E   M   A   K   A   A   G   L   A   T   G   N   V   S
1219 GAA ATG GCA AAA GCC GCA GGT CTG GCG ACC GGT AAC GTT TCT
     CTT TAC CGT TTT CGG CGT CCA GAC CGC TGG CCA TTG CAA AGA

+1   T   A   E   L   Q   D   A   T   P   A   A   L   V   A
1261 ACC GCA GAG TTG CAG GAT GCC ACG CCC GCT GCG CTG GTG GCA
     TGG CGT CTC AAC GTC CTA CGG TGC GGG CGA CGC GAC CAC CGT

+1   H   V   T   S   R   K   C   Y   G   P   S   A   T   S
1303 CAT GTG ACC TCG CGC AAA TGC TAC GGT CCG AGC GCG ACC AGT
     GTA CAC TGG AGC GCG TTT ACG ATG CCA GGC TCG CGC TGG TCA

+1   E   K   C   P   G   N   A   L   E   K   G   G   K   G
1345 GAA AAA TGT CCG GGT AAC GCT CTG GAA AAA GGC GGA AAA GGA
     CTT TTT ACA GGC CCA TTG CGA GAC CTT TTT CCG CCT TTT CCT

+1   S   I   T   E   Q   L   L   N   A   R   A   D   V   T
1387 TCG ATT ACC GAA CAG CTG CTT AAC GCT CGT GCC GAC GTT ACG
     AGC TAA TGG CTT GTC GAC GAA TTG CGA GCA CGG CTG CAA TGC

+1   L   G   G   G   A   K   T   F   A   E   T   A   T   A
1429 CTT GGC GGC GGC GCA AAA ACC TTT GCT GAA ACG GCA ACC GCT
     GAA CCG CCG CCG CGT TTT TGG AAA CGA CTT TGC CGT TGG CGA

+1   G   E   W   Q   G   K   T   L   R   E   Q   A   Q   A
1471 GGT GAA TGG CAG GGA AAA ACG CTG CGT GAA CAG GCA CAG GCG
     CCA CTT ACC GTC CCT TTT TGC GAC GCA CTT GTC CGT GTC CGC

```

FIG. 29-3

U.S. Patent

Apr. 25, 2006

Sheet 49 of 61

US 7,033,590 B1

```

+1 R G Y Q L V S D A A S L N S
1513 CGT GGT TAT CAG TTG GTG AGC GAT GCT GCC TCA CTG AAT TCG
    GCA CCA ATA GTC AAC CAC TCG CTA CGA CGG AGT GAC TTA AGC

+1 V T E A N Q Q K P L L G L F
1555 GTG ACG GAA GCG AAT CAG CAA AAA CCC CTG CTT GGC CTG TTT
    CAC TGC CTT CGC TTA GTC GTT TTT GGG GAC GAA CCG GAC AAA

+1 A D G N M P V R W L G P K A
1597 GCT GAC GGC AAT ATG CCA GTG CGC TGG CTA GGA CCG AAA GCA
    CGA CTG CCG TTA TAC GGT CAC GCG ACC GAT CCT GGC TTT CGT

+1 T Y H G N I D K P A V T C T
1639 ACG TAC CAT GGC AAT ATC GAT AAG CCC GCA GTC ACC TGT ACG
    TGC ATG GTA CCG TTA TAG CTA TTC GGG CGT CAG TGG ACA TGC

+1 P N P Q R N D S V P T L A Q
1681 CCA AAT CCG CAA CGT AAT GAC AGT GTA CCA ACC CTG GCG CAG
    GGT TTA GGC GTT GCA TTA CTG TCA CAT GGT TGG GAC CGC GTC

+1 M T D K A I E L L S K N E K
1723 ATG ACC GAC AAA GCC ATT GAA TTG TTG AGT AAA AAT GAG AAA
    TAC TGG CTG TTT CGG TAA CTT AAC AAC TCA TTT TTA CTC TTT

+1 G F F L Q V E G A S I D K Q
1765 GGC TTT TTC CTG CAA GTT GAA GGT GCG TCA ATC GAT AAA CAG
    CCG AAA AAG GAC GTT CAA CTT CCA CGC AGT TAG CTA TTT GTC

+1 D H A A N P C G Q I G E T V
1807 GAT CAT GCT GCG AAT CCT TGT GGG CAA ATT GGC GAG ACG GTC
    CTA GTA CGA CGC TTA GGA ACA CCC GTT TAA CCG CTC TGC CAG

+1 D L D E A V Q R A L E F A K
1849 GAT CTC GAT GAA GCC GTA CAA CGG GCG CTG GAA TTC GCT AAA
    CTA GAG CTA CTT CGG CAT GTT GCC CGC GAC CTT AAG CGA TTT

+1 K E G N T L V I V T A D H A
1891 AAG GAG GGT AAC ACG CTG GTC ATA GTC ACC GCT GAT CAC GCC
    TTC CTC CCA TTG TGC GAC CAG TAT CAG TGG CGA CTA GTG CGG

+1 H A S Q I V A P D T K A P G
1933 CAC GCC AGC CAG ATT GTT GCG CCG GAT ACC AAA GCT CCG GGC
    GTG CGG TCG GTC TAA CAA CGC GGC CTA TGG TTT CGA GGC CCG

+1 L T Q A L N T K D G A V M V
1975 CTC ACC CAG GCG CTA AAT ACC AAA GAT GGC GCA GTG ATG GTG
    GAG TGG GTC CGC GAT TTA TGG TTT CTA CCG CGT CAC TAC CAC

```

FIG. 29-4

U.S. Patent

Apr. 25, 2006

Sheet 50 of 61

US 7,033,590 B1

```

+1  M  S  Y  G  N  S  E  E  D  S  Q  E  H  T
2017 ATG AGT TAC GGG AAC TCC GAA GAG GAT TCA CAA GAA CAT ACC
    TAC TCA ATG CCC TTG AGG CTT CTC CTA AGT GTT CTT GTA TGG

+1  G  S  Q  L  R  I  A  A  Y  G  P  H  A  A
2059 GGC AGT CAG TTG CGT ATT GCG GCG TAT GGC CCG CAT GCC GCC
    CCG TCA GTC AAC GCA TAA CGC CGC ATA CCG GGC GTA CGG CGG

+1  N  V  V  G  L  T  D  Q  T  D  L  F  Y  T
2101 AAT GTT GTT GGA CTG ACC GAC CAG ACC GAT CTC TTC TAC ACC
    TTA CAA CAA CCT GAC TGG CTG GTC TGG CTA GAG AAG ATG TGG

+1  M  K  A  A  L  G  D  I  A  His tag
2143 ATG AAA GCC GCT CTG GGG GAT ATC GCA CAC CAT CAC CAT CAC
    TAC TTT CGG CGA GAC CCC CTA TAG CGT GTG GTA GTG GTA GTG

+1  H  *
2185 CAT TAA
    GTA ATT

```

**FIG. 29-5**



U.S. Patent

Apr. 25, 2006

Sheet 51 of 61

US 7,033,590 B1

PelB-Leader

```

+1  M  K  Y  L  L  P  T  A  A  A  G  L  L  L  L
1   ATG AAA TAC CTA TTG CCT ACG GCA GCC GCT GGA TTG TTA TTA CTC
    TAC TTT ATG GAT AAC GGA TGC CGT CGG CGA CCT AAC AAT AAT GAG

```

VH

```

+1  A  A  Q  P  A  M  A  E  V  Q  L  Q  Q  S  G
46  GCG GCC CAG CCG GCC ATG GCG GAG GTT CAG CTT CAG CAG TCT GGA
    CGC CGG GTC GGC CGG TAC CGC CTC CAA GTC GAA GTC GTC AGA CCT

```

```

+1  P  E  L  V  K  P  G  A  S  V  K  I  S  C  K
91  CCT GAG CTG GTG AAG CCC GGG GCC TCA GTG AAG ATT TCC TGC AAA
    GGA CTC GAC CAC TTC GGG CCC CGG AGT CAC TTC TAA AGG ACG TTT

```

```

+1  A  S  G  Y  A  F  S  S  S  W  M  N  W  V  K
136 GCT TCT GGC TAC GCA TTC AGT AGC TCT TGG ATG AAC TGG GTG AAG
    CGA AGA CCG ATG CGT AAG TCA TCG AGA ACC TAC TTG ACC CAC TTC

```

```

+1  Q  R  P  G  Q  G  L  E  W  I  G  R  I  Y  P
181 CAG AGG CCT GGA CAG GGT CTT GAG TGG ATT GGA CGG ATT TAT CCT
    GTC TCC GGA CCT GTC CCA GAA CTC ACC TAA CCT GCC TAA ATA GGA

```

```

+1  G  N  G  D  T  N  Y  N  G  K  F  K  G  K  A
226 GGA AAT GGA GAT ACT AAC TAC AAT GGG AAG TTC AAG GGC AAG GCC
    CCT TTA CCT CTA TGA TTG ATG TTA CCC TTC AAG TTC CCG TTC CGG

```

```

+1  T  L  T  A  D  K  S  S  S  T  A  Y  M  Q  L
271 ACA CTG ACT GCA GAC AAA TCC TCC AGC ACA GCC TAC ATG CAG CTC
    TGT GAC TGA CGT CTG TTT AGG AGG TCG TGT CGG ATG TAC GTC GAG

```

```

+1  S  S  L  T  S  V  D  S  A  V  Y  F  C  A  D
316 AGC AGC CTG ACC TCT GTG GAC TCT GCG GTC TAT TTC TGT GCA GAT
    TCG TCG GAC TGG AGA CAC CTG AGA CGC CAG ATA AAG ACA CGT CTA

```

```

+1  G  N  V  Y  Y  Y  A  M  D  Y  W  G  Q  G  T
361 GGT AAC GTA TAT TAC TAT GCT ATG GAC TAC TGG GGT CAA GGA ACC
    CCA TTG CAT ATA ATG ATA CGA TAC CTG ATG ACC CCA GTT CCT TGG

```

Linker

```

+1  S  V  T  V  S  S  G  G  G  G  S  G  G  R  A
406 TCA GTC ACC GTC TCC TCA GGT GGA GGC GGT TCA GGT GGG CGC GCC
    AGT CAG TGG CAG AGG AGT CCA CCT CCG CCA AGT CCA CCC GCG CGG

```

FIG. 30-1

US 7,033,590 B1

**FIG. 30-2**

**U.S. Patent**

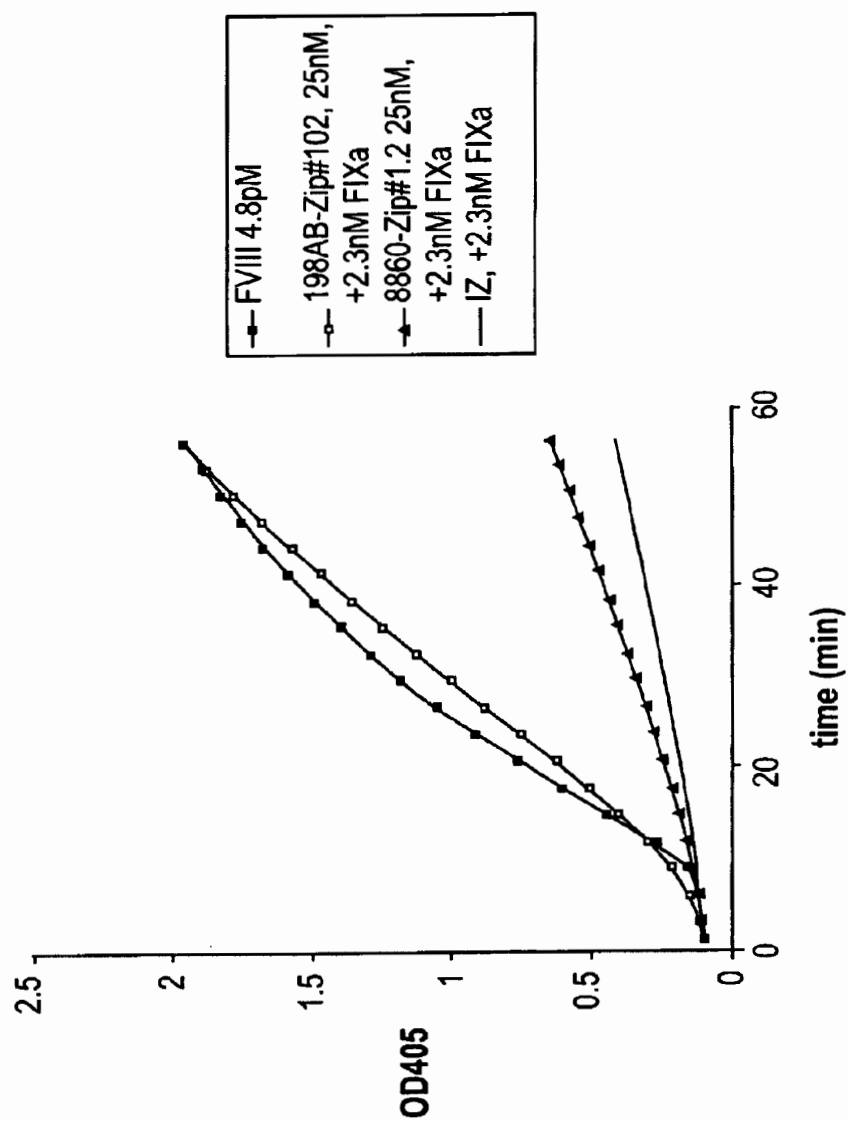
Apr. 25, 2006

Sheet 53 of 61

**US 7,033,590 B1**

Spacer		His-tag						
	+1 G	H	H	H	H	H	H	*
946	GGT	CAC	CAT	CAC	CAT	CAC	CAT	TAA
	CCA	GTG	GTA	GTG	GTA	GTG	GTA	ATT

**FIG. 30-3**

**FIG. 31**



U.S. Patent

Apr. 25, 2006

Sheet 56 of 61

US 7,033,590 B1

HindIII

2206 CAG GAA ACA GCT ATG ACC ATG ATT ACG CCA<sup>~</sup>AGC<sup>~</sup>TTC CAT GAA AAT  
GTC CTT TGT CGA TAC TGG TAC TAA TGC GGT TCG AAG GTA CTT TTA

PelB-Leader

M K Y L L P T

2251 TCT ATT TCA AGG AGA CAG TCA TAA TGA AAT ACC TAT TGC CTA CGG  
AGA TAA AGT TCC TCT GTC AGT ATT ACT TTA TGG ATA ACG GAT GCC

A A A G L L L L A A Q P A M A

SfiI

2296 CAG CCG CTG GAT TGT TAT TAC TCG CGG<sup>~</sup>CCC<sup>~</sup>AGC<sup>~</sup>CGG<sup>~</sup>CCA TGG CCC  
GTC GGC GAC CTA ACA ATA ATG AGC GCC GGG TCG GCC GGT ACC GGG

Polylinker

Q V Q L Q A R L Q V D L E I K

AscI

2341 AGG TGC AGC TGC AGG<sup>~</sup>CGC<sup>~</sup>GCC<sup>~</sup>TGC AGG TCG ACC TCG AGA TCA AAC  
TCC ACG TCG ACG TCC GCG CGG ACG TCC AGC TGG AGC TCT AGT TTG

Spacer Myc-tag

R A A A E Q K L I S E E D L N

NotI

2386 GGG<sup>~</sup>CGG<sup>~</sup>CCG<sup>~</sup>CAG AAC AAA AAC TCA TCT CAG AAG AGG ATC TGA ATG  
CCC GCC GGC GTC TTG TTT TTG AGT AGA GTC TTC TCC TAG ACT TAC

Spacer His tag

G A A H H H H H \* \*

EcoRI

2431 GGG<sup>~</sup>CGG<sup>~</sup>CAC ATC ACC ATC ACC ATC ACT AAT AAG<sup>~</sup>AAT<sup>~</sup>TCA CTG GCC  
CCC GCC GTG TAG TGG TAG TGG TAG TGA TTA TTC TTA AGT GAC CGG

FIG. 33

U.S. Patent

Apr. 25, 2006

Sheet 57 of 61

US 7,033,590 B1

PelB-leader

```

+1  M   K   Y   L   L   P   T   A   A   A   G   L   L   L   L
1   ATG AAA TAC CTA TTG CCT ACG GCA GCC GCT GGA TTG TTA TTA CTC
    TAC TTT ATG GAT AAC GGA TGC CGT CGG CGA CCT AAC AAT AAT GAG

+1  A   A   Q   P   A   M   A   |   VH
46  GCG GCC CAG CCG GCC ATG GCC | E   V   K   L   V   E   S   G
    CGC CGG GTC GGC CGG TAC CGG | CTC CAC TTC GAC CAC CTC AGA CCC

+1  G   G   L   V   K   P   G   G   S   L   K   L   S   C   A
91  GGA GGC TTA GTG AAG CCT GGA GGG TCC CTG AAA CTC TCC TGT GCA
    CCT CCG AAT CAC TTC GGA CCT CCC AGG GAC TTT GAG AGG ACA CGT

+1  A   S   G   F   T   F   S   S   Y   T   M   S   W   V   R
136 GCC TCT GGA TTC ACT TTC AGT AGC TAT ACC ATG TCT TGG GTT CGC
    CGG AGA CCT AAG TGA AAG TCA TCG ATA TGG TAC AGA ACC CAA GCG

+1  Q   T   P   E   K   R   L   E   W   V   A   T   I   S   S
181 CAG ACT CCG GAG AAG AGG CTG GAG TGG GTC GCA ACC ATT AGT AGT
    GTC TGA GGC CTC TTC TCC GAC CTC ACC CAG CGT TGG TAA TCA TCA

+1  G   G   S   S   T   Y   Y   P   D   S   V   K   G   R   F
226 GGN GGT AGT TCC ACC TAC TAT CCA GAC AGT GTG AAG GGC CGA TTC
    CCN CCA TCA AGG TGG ATG ATA GGT CTG TCA CAC TTC CCG GCT AAG

+1  T   I   S   R   D   N   A   K   N   T   L   Y   L   Q   M
271 ACC ATC TCC AGA GAC AAT GCC AAG AAC ACC CTG TAC CTG CAA ATG
    TGG TAG AGG TCT | CTG TTA CGG TTC TTG TGG GAC ATG GAC GTT TAC

+1  S   S   L   R   S   E   D   T   A   M   Y   Y   C   T   R
316 AGC AGT CTG AGG TCT GAG GAC ACA GCC ATG TAT TAC TGT ACA AGA
    TCG TCA GAC TCC AGA CTC CTG TGT CGG TAC ATA ATG ACA TGT TCT

+1  E   G   G   G   F   T   V   N   W   Y   F   D   V   W   G
361 GAG GGG GGT GGT TTC ACC GTC AAC TGG TAC TTC GAT GTC TGG GGC
    CTC CCC CCA CCA AAG TGG CAG TTG ACC ATG AAG CTA CAG ACC CCG

+1  A   G   T   S   V   T   V   S   S   |   Leader
406 GCA GGA ACC TCA GTC ACC GTC TCC TCA | G   G   G   G   S   G
    CGT CCT TGG AGT CAG TGG CAG AGG AGT | CCA CCT CCG CCA AGT CCA

```

FIG. 34-1

US 7,033,590 B1

**FIG. 34-2**



U.S. Patent

Apr. 25, 2006

Sheet 59 of 61

US 7,033,590 B1

Pel-leader

```

+1 M K Y L L P T A A A G L L L L
1  ATG AAA TAC CTA TTG CCT ACG GCA GCC GCT GGA TTG TTA TTA CTC
   TAC TTT ATG GAT AAC GGA TGC CGT CGG CGA CCT AAC AAT AAT GAG

```

VH

```

+1 A A Q P A M A E V Q L Q Q S G
46 GCG GCC CAG CCG GCC ATG GCC GAG GTT CAG CTT CAG CAG TCT GGA
   CGC CGG GTC GGC CGG TAC CGG CTC CAA GTC GAA GTC GTC AGA CCT

```

```

+1 P E L V K P G A S V K I S C K
91 CCT GAG CTG GTG AAG CCC GGG GCC TCA GTG AAG ATT TCC TGC AAA
   GGA CTC GAC CAC TTC GGG CCC CGG AGT CAC TTC TAA AGG ACG TTT

```

```

+1 A S G Y A F S S S W M N W V K
136 GCT TCT GGC TAC GCA TTC AGT AGC TCT TGG ATG AAC TGG GTG AAG
   CGA AGA CCG ATG CGT AAG TCA TCG AGA ACC TAC TTG ACC CAC TTC

```

```

+1 Q R P G Q G L E W I G R I Y P
181 CAG AGG CCT GGA CAG GGT CTT GAG TGG ATT GGA CGG ATT TAT CCT
   GTC TCC GGA CCT GTC CCA GAA CTC ACC TAA CCT GCC TAA ATA GGA

```

```

+1 G N G D T N Y N G K F K G K A
226 GGA AAT GGA GAT ACT AAC TAC AAT GGG AAG TTC AAG GGC AAG GCC
   CCT TTA CCT CTA TGA TTG ATG TTA CCC TTC AAG TTC CCG TTC CGG

```

```

+1 T L T A D K S S S T A Y M Q L
271 ACA CTG ACT GCA GAC AAA TCC TCC AGC ACA GCC TAC ATG CAG CTC
   TGT GAC TGA CGT CTG TTT AGG AGG TCG TGT CGG ATG TAC GTC GAG

```

```

+1 S S L T S V D S A V Y F C A D
316 AGC AGC CTG ACC TCT GTG GAC TCT GCG GTC TAT TTC TGT GCA GAT
   TCG TCG GAC TGG AGA CAC CTG AGA CGC CAG ATA AAG ACA CGT CTA

```

```

+1 G N V Y Y Y A M D Y W G Q G T
361 GGT AAC GTA TAT TAC TAT GCT ATG GAC TAC TGG GGT CAA GGA ACC
   CCA TTG CAT ATA ATG ATA CGA TAC CTG ATG ACC CCA GTT CCT TGG

```

Leader

```

+1 S V T V S S G G G S G G R A
406 TCA GTC ACC GTC TCC TCA GGT GGA GGC GGT TCA GGT GGG CGC GCC
   AGT CAG TGG CAG AGG AGT CCA CCT CCG CCA AGT CCA CCC GCG CGG

```

VL

```

+1 S G G G G S Q I V L T Q S P A
451 TCT GGC GGT GGC GGA TCG CAA ATT GTT CTC ACC CAG TCT CCT GCT
   AGA CCG CCA CCG CCT AGC GTT TAA CAA GAG TGG GTC AGA GGA CGA

```

FIG. 35-1

U.S. Patent

Apr. 25, 2006

Sheet 60 of 61

US 7,033,590 B1

```

+1 S L A V S L G Q R A T I S C R
496 TCC TTA GCT GTA TCT CTG GGG CAG AGG GCC ACC ATC TCA TGC AGG
    AGG AAT CGA CAT AGA GAC CCC GTC TCC CGG TGG TAG AGT ACG TCC

+1 A S K S V S T S G Y S Y M H W
541 GCC AGC AAA AGT GTC AGT ACA TCT GGC TAT AGT TAT ATG CAC TGG
    CGG TCG TTT TCA CAG TCA TGT AGA CCG ATA TCA ATA TAC GTG ACC

+1 Y Q Q K P G Q P P K L L I Y L
586 TAC CAA CAG AAA CCA GGA CAG CCA CCC AAA CTC CTC ATC TAT CTT
    ATG GTT GTC TTT GGT CCT GTC GGT GGG TTT GAG GAG TAG ATA GAA

+1 A S N L E S G V P A R F S G S
631 GCA TCC AAC CTA GAA TCT GGG GTC CCT GCC AGG TTC AGT GGC AGT
    CGT AGG TTG GAT CTT AGA CCC CAG GGA CGG TCC AAG TCA CCG TCA

+1 G S G T D F T L N I H P V E E
676 GGG TCT GGG ACA GAC TTC ACC CTC AAC ATC CAT CCT GTG GAG GAG
    CCC AGA CCC TGT CTG AAG TGG GAG TTG TAG GTA GGA CAC CTC CTC

+1 E D A A T Y Y C Q H S R E L P
721 GAG GAT GCT GCA ACC TAT TAC TGT CAG CAC AGT AGG GAG CTT CCT
    CTC CTA CGA CGT TGG ATA ATG ACA GTC GTG TCA TCC CTC GAA GGA

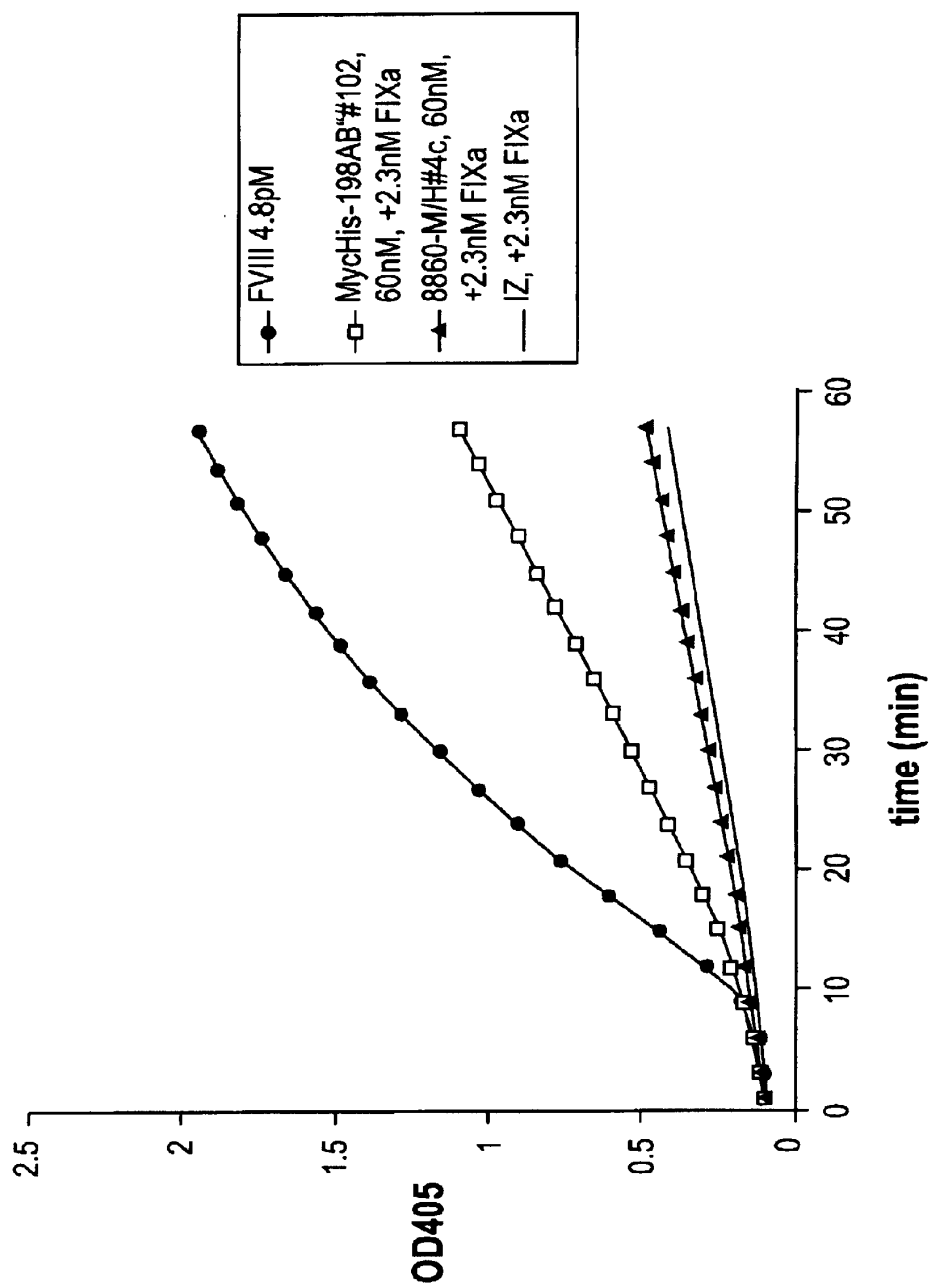
+1 R T F G G G T K L E I K R | Spacer
766 CGG ACG TTC GGT GGA GGC ACC AAG CTG GAA ATC AAA CGG | GGG GCC
    GCC TGC AAG CCA CCT CCG TGG TTC GAC CTT TAG TTT GCC | CGC CGG

+1 A | Myc-tag | Spacer
811 GCA | E Q K L I S E E D L N G | A A
    CGT | CTT GTT TTT GAG TAG AGT CTT CTC CTA GAC TTA CCC | CGC CGT

+1 H H H H H H *
856 CAT CAC CAT CAC CAT CAC TAA
    GTA GTG GTA GTG GTA GTG ATT

```

FIG. 35-2

**FIG. 36**

US 7,033,590 B1

1

# **FACTOR IX/FACTOR IXA ACTIVATING ANTIBODIES AND ANTIBODY DERIVATIVES**

The present invention relates to actor IX/factor IXa-antibodies and antibody derivatives.

Blood clots (thrombi) are formed by a series of zymogen activations referred to as the coagulation cascade. In the course of this enzymatic cascade, the activated form of each of such zymogens (referred to as factors) catalyzes the activation of the next one. Thrombi are deposits of blood components on the surface of a blood vessel wall and mainly consist of aggregated blood platelets and insoluble, cross-linked fibrin. Fibrin formation is effected by means of thrombin by limited proteolysis of fibrinogen. Thrombin is the final product of the coagulation cascade, (K. G. Mann, Blood, 1990, Vol. 76, pp. 1-16).

Activation of factor x by the complex of activated factor IX (FIXa) and activated factor VIII (FVIIIa) is a key step in coagulation. The absence of the components of this complex or a disturbance of their function is associated with the blood coagulation disorder called hemophilia (J. E. Sadler & E. W. Davie: Hemophilia A, Hemophilia B and von Willebrand's disease, in G. Stamatoyannopoulos et al. (Eds.): The molecular basis of blood diseases. W. B. Saunders Co., Philadelphia, 1987, pp. 576-602). Hemophilia A denotes a (functional) absence of factor VIII activity, while Hemophilia B is characterized by the absence of factor IX activity. At present, treatment of Hemophilia A is effected via a substitution therapy by administering factor VIII concentrates. However, approximately 20-30% of Hemophilia A patients develop factor VIII inhibitors (i.e. antibodies against factor VIII), whereby the effect of administered factor VIII preparations is inhibited. Treatment of factor VIII inhibitor patients is very difficult and involves risks, and so far there exist only a limited number of treatments for these patients.

In the case of patients having a low FVIII inhibitor level, it is possible, though expensive, to administer high doses of factor VIII to such patients and thus to neutralize the antibodies against factor VIII. The amount of factor VIII beyond that needed to neutralize the inhibitor antibodies then has hemostatic action. In many cases, desensitization can be effected, whereupon it is then possible again to apply standard factor VIII treatments. Such high dose factor VIII treatments require, however, large amounts of factor VIII, are time-consuming and may involve severe anaphylactic side reactions. Alternatively, the treatment may be carried out with porcine factor VIII molecules.

A further high-cost method involves removing factor VIII inhibitors through extra corporeal immunoadsorption on lectins which bind to immunoglobulins (protein A, protein G) or to immobilized factor VIII. Since the patient must be connected to an apheresis machine during this treatment, the treatment also constitutes a great burden on the patient. It is also not possible to treat an acute hemorrhage in this way.

At present, the therapy of choice is to administer activated prothrombin complex concentrates (APCC), such as FEIBA® and AUTOPLEX®, which are suitable for the treatment of acute hemorrhages even in patients having a high inhibitor titer (DE 31 27 318).

In the intravascular system of blood coagulation, the last step is the activation of factor X. This reaction is stimulated by the binding of factor VIIIa to factor IXa and the formation of a "tenase"-complex consisting of the factors IXa, VIIIa, X and phospholipid. Without the binding of FVIIIa, FIXa exhibits no or only a very slight enzymatic activity relative to FX.

2

Over the last several years, a number of possible binding sites for factor VIIIa to factor IXa have been characterized, and it has been shown that antibodies or peptides which bind to these regions inhibit the activity of FIXa (Fay et al., J. Biol. Chem., 1994, Vol. 269, pp. 20522-20527, Lenting et al., J. Biol. Chem., 1996, Vol. 271, pp. 1935-1940, Jorquera et al., Circulation, 1992, Vol. 86, Abstract 2725). The inhibition of coagulation factors, such as factor IX, has also been achieved through the use of monoclonal antibodies with the aim of preventing thrombosis formation (WO 97/26010).

The opposite effect, i.e. an increase in the factor IXa mediated activation of factor X, has been described by Liles D. K. et al., (Blood, 1997, Vol. 90, suppl. 1, 2054) through the binding of a factor VIII peptide (amino acids 698-712) to factor IX. Yet, this effect only occurs in the absence of factor VIIIa, while in the presence of factor VIIIa the factor IXa/factor VIIIa-mediated cleavage of factor X is inhibited by this peptide.

## **SUMMARY OF THE INVENTION**

With a view to the possible risks and side effects which may occur in the treatment of hemophilia patients, there is a need for a therapy which allows for the effective treatment of FVIII inhibitor patients. Therefore, it is an object of the present invention to provide a preparation for the treatment of blood coagulation disorders which has particular advantages for factor VIII inhibitor patients.

According to the present invention, this object is achieved through the use of antibodies or antibody derivatives against factor IX/factor IXa which have factor VIIIa-cofactor activity or factor IXa-activating activity and lead to an increase in the procoagulant activity of factor IXa. Surprisingly, the action of these inventive factor IX/factor IXa-activating antibodies and antibody derivatives is not negatively affected by the presence of inhibitors, such as inhibitors against factor VIII/factor VIIIa, but instead the procoagulant activity of factor IXa in this case also is increased.

A further advantage of this invention is that the administration of the preparation according to the invention allows for rapid blood coagulation even in the absence of factor VIII or factor VIIIa, even in the case of FVIII inhibitor patients. Surprisingly, these agents are also effective in the presence of factor VIIIa.

The antibodies and antibody derivatives according to the present invention thus have a FVIII-cofactor-like activity which, in a FVIII assay (e.g. a COATEST® assay or Immunochrom test) after 2 hours of incubation exhibits a ratio of background (basic noise) to measured value of at least 3. Calculation of this ratio may, e.g., be effected according to the following scheme:

$$\frac{\text{Antibody measurement (OD 405)} - \text{blank value from reagent}}{\text{Mouse-IgG-measurement (OD 405)} - \text{blank value from reagent}} \geq 3$$

after two hours of incubation.

The antibodies according to the invention preferably have an in vivo half life of at least 5 days, more preferably at least 10 days, though it is more preferred to have a half life of at least 20 days.

A further aspect of this invention is a preparation comprising antibodies and/or antibody derivatives against factor IX/factor IXa and a pharmaceutically acceptable carrier substance. Furthermore, the preparation according to the invention may additionally comprise factor IX and/or factor IXa.

US 7,033,590 B1

3

A further aspect of the invention is the use of the antibodies or antibody derivatives to increase the amidolytic activity of factor IXa.

FIG. 1 shows the results of a screening of supernatants from hybridoma cell cultures for FVIII-like activity. Pre-selected clones from fusion experiments, #193, #195 and #196, were tested in a chromogenic assay.

FIG. 2 shows the results of screening for IgG-mediated factor VIII-like activity in supernatants of a hybridoma cell culture of a master plate.

FIG. 3 shows the subcloning of clone 193/C0, namely the results of the first cloning round.

FIG. 4 shows a comparison of the chromogenic FVIII-like activity and factor IX-ELISA-reactivity of hybridoma cultures derived from the starting clone 193/C0.

FIG. 5 shows the results of the measurement of the chromogenic activity of some master clones and sub-clones.

FIG. 6A shows the FVIII-like activity of the anti-FIX/FIXa-antibodies 193/AD3 and 196/AF2 compared to human FVIII, TBS buffer and cell culture medium. After a lag phase, both antibodies gave rise to chromogenic substrate cleavage, as judged by the increasing optical density.

FIG. 6B shows a comparison of the chromogenic activity of factor VIII, 196/AF1, 198/AC1/1 and mouse-IgG.

FIG. 7A shows a comparison of the kinetics of Factor Xa generation by Factor VIII and 196/AF2 with and without the addition of a Factor Xa specific inhibitor.

FIG. 7B shows a comparison of the kinetics of the Factor Xa generation by Factor VIII, mouse-IgG and anti-factor IX/IXa-antibody 198/AM1 with and without the addition of a factor Xa-specific inhibitor, Pefabloc Xa®.

FIG. 8A shows a measurement of the dependence of the factor VIII-like activity of purified anti-factor IX/IXa-antibody 198/AC1/1 in the presence and absence of phospholipids, FIXa/FX and calcium ions.

FIG. 8B shows a measurement of the dependence of FXa generation by anti-FIXa-antibody 196/AF1 in the presence of phospholipids, Ca<sup>2+</sup> in FIXa/FX.

FIG. 8C shows the generation of FXa by unspecific mouse IgG antibody.

FIG. 9 is a graphical representation of the coagulation times of Factor VIII-deficient plasma in an APTT assay by using various concentrations of anti-factor IX/IXa-antibody 193/AD3.

FIG. 10A shows that in the presence of Factor IXa, antibody 193/AD3 leads to a reduction in the coagulation time of factor VIII-deficient plasma.

FIG. 10B shows a dose-dependent reduction of the clotting time by antibody 193/AD3 in the presence of factor IXa- and factor VIII-inhibitors.

FIG. 11 shows the chromogenic activity of antibodies 198/A1, 198/B1 and 198/AP1 in the presence and absence of human FIXaβ.

FIG. 12 shows the primer sequences (SEQ ID NOS:50-61) for the amplification of the genes of the variable heavy chain of mouse antibody.

FIG. 13 shows the primer sequences (SEQ ID NOS:65-78) for the amplification of the genes of the variable light (kappa) chain of the mouse antibody.

FIG. 14 shows the DNA and derived protein sequence of the scFv from hybridoma cell line 193/AD3 (SEQ.ID.NOS. 81 and 82).

FIG. 15 shows the DNA and derived protein sequence of the scFv from hybridoma cell line 193/K2 (SEQ.ID.NOS. 83 and 84).

FIG. 16 shows the DNA and derived protein sequence of the scFv from hybridoma cell line 198/AB2 (subclone of 198/B1) (SEQ.ID.NOS. 85 and 86).

4

FIG. 17 shows the DNA and deduced protein sequence of scFv derived from the cell line 198/A1 (SEQ.ID.NOS. 87 and 88).

FIG. 18 demonstrates the chromogenic FVIII-like activity of peptide A1/3 in the presence of 2.9 nM human FIXa. The scrambled version of peptide A1/3, peptide A1/5 does not give rise to any FXa generation.

FIG. 19 demonstrates the dependence of the chromogenic FVIII-like activity of peptide A1/3 on the presence of human FIXa. In the absence of human FIXa, peptide A1/3 does not give rise to any FXa generation. The buffer control, plain imidazole buffer is designated IZ.

FIG. 20 shows that the chirality of Arg-residues does not play a significant role for the chromogenic activity of peptides A1/3-rd and A1/3-Rd-srmb.

FIG. 21 shows that the addition of 2.4 μM peptide B1/7 to the reaction mixture led to a measureable generation of FXa.

FIG. 22 shows that the addition of a FX-specific inhibitor results in a significant reduction in the reaction. If there was no FIXa and FX is added to the reaction mixture, no FXa was synthesized.

FIG. 23 shows vector pBax-IgG1.

FIG. 24 shows the increase of the amidolytic activity of FIXa in the presence of antibody 198/B1 (FIG. 24A) and IgM antibody 198/AF1 (FIG. 24B).

FIG. 25 demonstrates the chromogenic FVIII-like activity of the antibody 198/A1 Fab fragment in the presence of 2.3 nM human FIXa. As a positive control the intact antibody 198/A1 was used as well as 7.5 pM FVIII. The buffer control (IZ) was used as a negative control.

FIG. 26 shows the nucleotide and amino acid sequence of the 198AB2 scFv-alkaline phosphatase fusion protein (ORF of the expression vector pDAP2-198AB2#100, (SEQ.ID.NOS. 89 and 90).

The genes for the VL and the VH domains of antibody 198/AB2 (198/AB2 is an identical subclone of 198/B1) were derived from the corresponding hybridoma cells as described in example 10. The PCR product of the VH-gene was digested SfiI-AscI and the PCR-product of the VL-gene was digested AscI and NotI. VH and VL genes were linked via the AscI site and inserted into SfiI-NotI digested vector pDAP2 (Kerschbaumer R. J. et al, Immuno-technology 2, 145-150, 1996; GeneBank accession No.:U35316). PelB leader: leader sequence of *Erwinia carotovora* Pectate Lyase B, His tag, Histidine tag for metal ion chromatography.

FIG. 27 demonstrates the chromogenic FVIII-like activity of two antibody 198/B1 (subclone AB2) scFv fragment-alkaline phosphatase fusion proteins (198AB2#1 and 198AB2#100) in the presence of 2.3 nM human FIXa. As a positive control 7.5 pM FVIII was used.

FIG. 28 shows the amino acid and nucleotide sequence of pZip198AB2#102 (SEQ.ID.NOS. 91 and 92).

FIG. 29 shows the nucleotide and amino acid sequence of the mAB#8860 scFv-alkaline phosphatase fusion protein (vector pDAP2-8860scFv#11, (SEQ.ID.NOS. 93 and 94). The genes for the VT and the VH domains of antibody #8860 were derived from the corresponding hybridoma cells as described in example 10. The PCR product of the VH-gene was digested SfiI-AscI and the PCR-product of the VL-gene was digested AscI and NotI. VH and VL genes were linked via the AscI site and inserted into SfiI-NotI digested vector pDAP2 (Kerschbaumer R. J. et al, Immuno-technology 2, 145-150, 1996; GeneBank accession No.:U35316).

FIG. 30 shows the nucleotide and amino acid sequence of the mAB #8860 scFv-leucine zipper fusion protein

US 7,033,590 B1

5

(miniantibody; vector p8860-Zip#1.2, (SEQ.ID.NOs. 95 and 96). The gene of the scFv fragment was derived from mAb #8860 and was swapped from vector pDAP2-8860scFv#11 into SfiI-NotI digested plasmid pZip1 (Kerschbaumer R. J. et al., Analytical Biochemistry 249, 219-227, 1997; GeneBank accession No.: U94951)

FIG. 31 demonstrates the chromogenic FVIII-like activity of the 198/B1 (subclone AB2) miniantibody 198AB-Zip#102 in the presence of 2.3 nM human FIXa. As a positive control 4.5 pM FVIII was used whereas a unrelated miniantibody (8860-Zip#1.2) and plain reaction buffer (IZ) served as negative controls.

FIG. 32 shows a schematic representation of the plasmid pMycHis6 (SEQ ID NOS:107-110).

FIG. 33 shows the nucleotide and amino acid sequence of the part of the plasmid pMycHis6 differing from vector pCOCK (SEQ.ID.Nos. 97 and 98). Vector pMycHis6 was constructed by cleaving vector pCOCK (Engelhardt et al., 1994, Biotechniques, 17: 44-46) with NotI and EcoRI and insertion of the oligonucleotides: mychis6-co: 5'ggccgca-gaacaactcatctcagaagaggatct gaatggggcgccacatccatcac-cateactaataag 3' (SEQ ID.No. 79) and mychis-ic: 5' aattct-tattagtgttggtgatggtgatgtgcgcgccattcagatcctcttct gagatgagttttgtctgc (SEQ.ID.No. 80).

FIG. 34 shows the nucleotide and amino acid sequence of 198AB2 scFv (linked to the c-myc-tag and the His6tag): ORF of the expression vector pMycHis6-198AB2#102. Vector pMycHis6 was constructed by cleaving vector pCOCK (Engelhardt O. et al, BioTechniques 17, 44-46, 1994) NotI-EcoRI and inserting the following annealed oligonucleotides: (5'-GGCCGCAGAACAAAACTCATCTCAGAA GAGGATCTGAATGGGGCGGCGCACATCA CCATCACCATCACTAATAAG-3' (SEQ.ID.No. 103) and 5'-TTATTAGTGATGGTGTATGGT GATGTGCC GCCCATTCAGATCCTTCTTGAGATGAGTTTGT TTCTGC-3'(SEQ.ID.NO. 104)). The resultant vector, named pMycHis6, was cleaved SfiI-NotI and the gene of scFv 198AB2 was swapped into this vector from vector pDAP2-198AB2#100.

FIG. 35 shows the nucleotide and amino acid sequence of the mAb #8860 scFv linked to the c-myc-tag and the His6-tag (vector p8860-M/H#4c, SEQ.ID.NOs. 101 and 102). Plasmid pMycHis6 was cleaved with SfiI and NotI and the DNA sequence coding for the scFv 8860#11 protein was inserted from pDAP2-8860scFv#11 (see FIG. 29) yielding plasmid p8860-M/H#4c.

FIG. 36 demonstrates the chromogenic FVIII-like activity of the 198/B1 (subclone AB2) scFv fragment (MycHis-198AB2#102) in the presence of 2.3 nM human FIXa. As a positive control 4.8 pM FVIII was used whereas a unrelated scFv (8860-M/H#4c) and plain reaction buffer (IZ) served as negative controls.

#### Antibodies and Antibody Derivatives

The present invention also comprises the nucleic acids encoding the inventive antibodies and antibody derivatives, expression vectors, hybridoma cell lines, and methods for producing the same.

Antibodies are immunoglobulin molecules having a specific amino acid sequence which only bind to antigens that induce their synthesis (or its immunogen, respectively) or to antigens (or immunogens) which are very similar to the former. Each immunoglobulin molecule consists of two types of polypeptide chains. Each molecule consists of large, identical heavy chains (H chains) and two light, also identical chains (L chains). The polypeptides are connected by disulfide bridges and non-covalent bonds. In vivo, the heavy and light chains are formed on different ribosomes, assembled in the cell, and secreted as intact immunoglobulins (Roitt I. et al., in: Immunology, second ed., 1989).

6

The inventive antibodies and antibody derivatives and organic compounds derived there from comprise human and animal monoclonal antibodies or fragments thereof, single chain antibodies and fragments thereof and miniantibodies, bispecific antibodies, diabodies, triabodies, or di-, oligo- or multimers thereof. Also included are peptidomimetics or peptides derived from the antibodies according to the invention, e.g. they comprise one or several CDR regions, preferably the CDR3 region.

Further included are human monoclonal antibodies and peptide sequences which, based on a structure activity connection, are produced through an artificial modeling process (Greer J. et al., J. Med. Chem., 1994, Vol. 37, pp. 1035-1054).

The term factor IX/IXa activating antibodies and antibody derivatives may also include proteins produced by expression of an altered, immunoglobulin-encoding region in a host cell, e.g. "technically modified antibodies" such as synthetic antibodies, chimeric or humanized antibodies, or mixtures thereof, or antibody fragments which partially or completely lack the constant region, e.g. Fv, Fab, Fab' or F(ab)<sub>2</sub> etc. In these technically modified antibodies, e.g., a part or parts of the light and/or heavy chain may be substituted. Such molecules may, e.g., comprise antibodies consisting of a humanized heavy chain and an unmodified light chain (or chimeric light chain), or vice versa. The terms Fv, Fc, Fd, Fab, Fab' or F(ab)<sub>2</sub> are used as described in the prior art (Harlow E. and Lane D., in "Antibodies, A Laboratory Manual", Cold Spring Harbor Laboratory, 1988).

The present invention also comprises the use of Fab fragments or F(ab)<sub>2</sub> fragments which are derived from monoclonal antibodies (mAb), which are directed against factor IX/factor IXa and cause an increase of the procoagulant activity of factor IXa.

Preferably, the heterologous framework regions and constant regions are selected from the human immunoglobulin classes and isotypes, such as IgG (subtypes 1 to 4), IgM, IgA and IgE. In the course of the immune response, a class switch of the immuno-globulins may occur, e.g. a switch from IgM to IgG; therein, the constant regions are exchanged, e.g. from p to y. A class switch may also be caused in a directed manner by means of genetic engineering methods ("directed class switch recombination"), as is known from the prior art (Esser C. and Radbruch A., Annu. Rev. Immunol., 1990, Vol. 8, pp. 717-735). However, the antibodies and antibody derivatives according to the present invention need not comprise exclusively human sequences of the immunoglobulin proteins.

In one particular embodiment, a humanized antibody comprises complement determining regions (CDRs) from murine monoclonal antibodies which are inserted in the framework regions of selected human antibody sequences. However, human CDR regions can also be used. Preferably, the variable regions in the human light and heavy chains are technically altered by one or more CDR exchanges. It is also possible to use all six CDRs or varying combinations of less than six CDRs.

The humanized antibody according to the present invention preferably has the structure of a human antibody or of a fragment thereof and comprises the combination of characteristics necessary for a therapeutic application, e.g., the treatment of coagulation disorders in patients, preferably factor VIII inhibitor patients.

A chimeric antibody differs from a humanized antibody in that it comprises the entire variable regions including the framework regions of the heavy and light chains of non-human origin in combination with the constant regions of

US 7,033,590 B1

7

both chains from human immuno-globulin. A chimeric antibody consisting of murine and human sequences may, for example, be produced. According to the present invention, the antibodies and antibody derivatives may also be single chain antibodies or miniantibodies (scFv fragments, which, e.g., are linked to proline-rich sequences and oligomerisation domains, e.g. Pluckthun A. and Pack P., Immunotechnology, 1997, Vol. 3, pp. 83–105) or single chain Fv (sFv) which incorporate the entire antibody binding region in one single polypeptide chain. For instance, single chain antibodies may be formed by linking the V-genes to an oligonucleotide which has been constructed as a linker sequence and connects the C terminus of the first V region with the N terminus of the second V region, e.g. in the arrangement VH-Linker-VL or VL-Linker-VH; both,  $V_H^{and}$  and  $V_L$  thus may represent the N-terminal domain (Huston JS et al., Int. Rev. Immunol., 1993, Vol. 10, pp. 195–217; Raag R. and Whitlow M., FASEB J., 1995, Vol. 9, pp. 73–80). The protein which can be used as linker sequence may, e.g., have a length of up to 150 Å, preferably up to 80 Å, and more preferably up to 40 Å. Linker sequences containing glycine and serine are particularly preferred for their flexibility, or glutamine and lysine, respectively, for their solubility. The choice of the amino acid is effected according to the criteria of immunogenicity and stability, also depending on whether or not these single chain antibodies are to be suitable for physiological or industrial applications (e.g. immunoaffinity chromatography). The single chain antibodies may also be present as aggregates, e.g. as trimers, oligomers or multimers. The linker sequence may, however, also be missing, and the connection of the  $V_H$  and  $V_L$  chains may occur directly.

Bispecific antibodies are macromolecular, heterobifunctional cross-linkers having two different binding specificities within one single molecule. In this group belong, e.g., bispecific (bs) IgGs, bs IgM-IgAs, bs IgA-dimers, bs (Fab')<sub>2</sub>, bs(scFv)<sub>2</sub>, diabodies, and bs bis Fab Fc (Cao Y. and Suresh M. R., Bioconjugate Chem., 1998, Vol. 9, pp. 635–644).

By peptidomimetics, protein components of low molecular weight are understood which imitate the structure of a natural peptide component, or of templates which induce a specific structure formation in an adjacent peptide sequence (Kemp DS, Trends Biotechnol., 1990, pp. 249–255). The peptidomimetics may, e.g., be derived from the CDR3 domains. Methodical mutational analysis of a given peptide sequence, i.e. by alanine or glutamic acid scanning mutational analysis, allows for the identification of peptide residues critical for procoagulant activity. Another possibility to improve the activity of a certain peptide sequence is the use of peptide libraries combined with high throughput screening.

The term antibodies and antibody derivatives may also comprise agents which have been obtained by analysis of data relating to structure-activity relationships. These compounds may also be used as peptidomimetics (Grassy G. et al., Nature Biotechnol., 1998, Vol. 16, pp. 748–752; Greer J. et al., J. Med. Chem., 1994, Vol. 37, pp. 1035–1054).

Examples of hybridoma cells expressing the antibodies or antibody derivatives according to the invention were deposited on 9 Sep. 1999 under the numbers 99090924 (#198/A1), 99090925 (#198/B1) and 99090926 (#198/BB1) and on Dec. 16, 1999 under the numbers 99121614 (#193/A0), 99121615 (#196/c4), 99121616 (#198/D1), 99121617 (198/T2), 99121618 (#198/G2), 99121619 (#198/AC1) and 99121620 (#198/U2) according to the Budapest Treaty.

#### Methods of Production:

The antibodies of the present invention can be prepared by methods known from the prior art, e.g. by conventional

8

hybridoma techniques, or by means of phage display gene libraries, immunoglobulin chain shuffling or humanizing techniques (Harlow E. and Lane D., in: Antibodies, A Laboratory Manual, Cold Spring Harbor Laboratory, 1988). The production of the inventive antibodies and antibody derivatives may, for instance, be made by conventional hybridoma techniques (Antibodies, A Laboratory Manual, Cold Spring Harbor Laboratory, 1988, Eds. Harlow and Lane, pp. 148–242). According to the present invention, human and also non-human species may be employed therefore, such as cattle, pigs, monkeys, chickens and rodents (mice, rats). Normal, immunocompetent Balb/c mice or FIX-deficient mice may, e.g., be used (factor IX-deficient mice may be obtained from Dr. Darrel Stafford from the University of North Carolina, Chapel Hill). Immunization may, e.g., be effected with factor IX, factor IXα or completely activated factor IXαβ, or with fragments thereof.

The hybridomas are selected with a view to the fact that the antibodies and antibody derivatives in the supernatants of the hybridoma cells bind to factor IX/factor IXα and cause an increase of the procoagulant activity of factor IXα. The increase in the procoagulant activity may, e.g., be proven by assaying methods as known from the prior art for the measurement of factor VIII-like activity, e.g. chromogenic assays.

Alternatively, the antibodies and antibody derivatives of the invention may also be produced by recombinant production methods. In doing so, the DNA sequence of the antibodies according to the invention can be determined by known techniques, and the entire antibody DNA or parts thereof can be expressed in suitable systems. Recombinant production methods can be used, such as those involving phage display, synthetic and natural libraries, expression of the antibody proteins in known expression systems, or expression in transgenic animals (Jones et al., Nature, 1986, Vol. 321, pp. 522–525; Phage Display of Peptides and Proteins, A Laboratory Manual, 1996, Eds. Kay et al., pp. 127–139; U.S. Pat. No. 4,873,316; Vaughan T. J. et al., Nature Biotechnology, 1998, pp. 535–539; Persic L. et al., Gene, 1997, pp. 9–18; Ames R. S. et al., J.Immunol.Methods, 1995, pp. 177–186).

The expression of recombinantly produced antibodies may be effected by means of conventional expression vectors, such as bacterial vectors, such as pBr322 and its derivatives, pSKF or eukaryotic vectors, such as pMSG and SV40 vectors. Those sequences which encode the antibody may be provided with regulatory sequences which regulate the replication, expression and secretion from the host cell. These regulatory sequences comprise promoters, e.g. CMV or SV40, and signal sequences.

The expression vectors may also comprise selection and amplification markers, such as the dihydrofolate reductase gene (DHFR), hygromycin-B phosphotransferase, thymidine-kinase etc.

The components of the vectors used, such as selection markers, replicons, enhancers etc., may either be commercially obtained or prepared by means of conventional methods. The vectors may be constructed for the expression in various cell cultures, e.g. for mammalian cells such as CHO, COS, fibroblasts, insect cells, yeast or bacteria, such as *E. coli*. Preferably, those cells are used which allow for an optimal glycosylation of the expressed protein. Particularly preferred is the vector pBax (cf. FIG. 17) which is expressed in CHO cells or in SK-Hep.

The production of Fab fragments or F(ab)<sub>2</sub> fragments may be effected according to methods known from the prior art, e.g. by cleaving a mAb with proteolytic enzymes, such as

US 7,033,590 B1

9

papain and/or pepsin, or by recombinant methods. These Fab and F(ab)<sub>2</sub> fragments may also be prepared by means of a phage display gene library (Winter et al., 1994, *Ann. Rev. Immunol.*, 12: 433-455).

The antibody derivatives may also be prepared by means of methods known from the prior art, e.g. by molecular modeling, e.g. from Grassy G. et al., *Nature Biotechnol.*, 1998, Vol. 16, pp. 748-752, or Greer J. et al., *J. Med. Chem.*, Vol. 37, pp. 1035-1054, or Rees A. et al., in: "Protein Structure Prediction: A practical approach", ed. Sternberg M. J. E., IRL press, 1996, chapt. 7-10, pp. 141-261.

The purification of the inventive antibodies and antibody derivatives may also be carried out by methods described in the prior art, e.g., by ammonium sulfate precipitation, affinity purification (protein G-Sepharose), ion exchange chromatography, or gel chromatography. The following methods may be used as the test methods to show that the antibodies and antibody derivatives of the present invention bind to factor IX/factor IXa, increase the procoagulant activity of factor IXa or have factor VIII-like activity: the one step coagulation test (Mikaelsson and Oswaldson, *Scand. J. Haematol.*, Suppl., 33, pp. 79-86, 1984) or the chromogenic tests, such as COATEST VIII:C® (Chromogenix) or Immunochrom (IMMUNO). In principle, all the methods used for determining factor VIII activity may be used. As the control blank value for the measurements, e.g., unspecific mouse-IgG antibody may be used.

The present antibodies and antibody derivatives are suitable for therapeutic use in the treatment of coagulation disorders, e.g. in the case of hemophilia A, for factor VIII inhibitor patients etc. Administration may be effected by any method suitable to effectively administer the therapeutic agent to the patient, e.g. by oral, subcutaneous, intramuscular, intravenous or intranasal administration.

Therapeutic agents according to the invention may be produced as preparations which comprise a sufficient amount of antibodies or of antibody derivatives as the active agent in a pharmaceutically acceptable carrier substance. These agents may be present either in liquid or in powdered form. Moreover, the preparations according to the invention may also comprise mixtures of different antibodies, the derivatives thereof and/or organic compounds derived therefrom, as well as mixtures consisting of antibodies and factor IX and/or factor IXa. Factor IXa may be present as factor IXaα and/or factor IXaβ. An example of an aqueous carrier substance is, e.g., saline. The solutions are sterile, sterilisation being effected by conventional methods.

The antibodies or antibody derivatives according to the invention may be present in lyophilized form for storage and be suspended in a suitable solvent before administration. This method has proven generally advantageous for conventional immunoglobulins, and known lyophilisation and reconstitution methods may be applied in this case.

Moreover, the antibodies and antibody derivatives according to the invention may also be used for industrial applications, e.g. for the purification of factor IX/factor IXa by means of affinity chromatography, or as a component of detection methods (e.g. ELISA assays), or as an agent for identification of and interaction with functional domains of a target protein.

The present invention will be described in more detail by way of the following examples and drawing figures, to which, however, it shall not be restricted.

#### EXAMPLES

##### Example 1

##### Immunization of Immunocompetent Mice and Generation of Anti-FIX/IXa Antibody Secreting Hybridoma Cells

Groups of 1-3 normal immunocompetent 5-8 week old Balb/c mice were immunized with 100 µg antigen (100 µl

10

doses) via the intraperitoneal (i.p.) route. In a typical experiment, mice were inoculated with either recombinant human coagulation factor (F) IX (Benefix™), human activated FIXαα (Enzyme Research Laboratories, Lot: FIXαα 1190L) or human FIXαβ (Enzyme Research Laboratories, Lot: HFIXAαβ 1332 AL,) adjuvanted with Al(OH)<sub>3</sub> or KFA.

Individual mice were boosted at various times with 100 µg antigen (10011 doses, i.p) and sacrificed two days later. Spleen cells were removed and fused to P3×63-Ag8 6.5.3 myeloma cells essentially as described by Lane et al., 1985 (*J. Immunol. Methods*, Vol. 81, pp. 223-228). Each fusion experiment was individually numbered, i.e. #193, 195, 196 or 198.

Hybridoma cells were grown in 96 well plates on a macrophage feeder layer (app. 10<sup>5</sup> cells/ml) and selected in HAT-medium (RPMI-1640 medium supplemented with antibiotics, 10% FCS, Na-pyruvate, L-glutamine, 2-mercaptoethanol and HAT (HAT 100x: 1.0×10<sup>-2</sup>M hypoxanthine in H<sub>2</sub>O (136.1 mg/100 ml H<sub>2</sub>O), 4.0×10<sup>-5</sup>M aminopterin in H<sub>2</sub>O (1.76 mg/100 ml H<sub>2</sub>O) and 1.6×10<sup>-3</sup>M thymidine in H<sub>2</sub>O (38.7 mg/100 ml H<sub>2</sub>O)). Medium was first changed after 6 days and thereafter twice a week. After 2-3 weeks HAT-medium was changed to HT-medium (RPMI-1640 supplemented with antibiotics, 10% FCS, Na-pyruvate, L-glutamine, 2-mercaptoethanol and HT) and later on (after additional 1-2 weeks) to normal growth medium (RPMI-1640 medium supplemented with 10% FCS, Na-pyruvate, L-glutamine and 2-mercaptoethanol) (see: HYBRIDOMA TECHNIQUES, EMBO, SKMB Course 1980, Base1).

In another set of experiments FIX deficient C57B16 mice (Lin et al., 1997, *Blood*, 90: 3962) were used for immunization and subsequent hybridoma production. Since FIX knockout (k.o.) mice do not express endogenous FIX, the anti (a)-FIX antibody spectrum achievable is supposed to be different compared to normal Balb/c mice (due to lack of tolerance).

##### Example 2

##### Assaying for FVIII-like Activity in Supernatants of Anti-Fix/FIXa Antibody Secreting Hybridoma Cells

In order to assay the FVIII-like activity of anti-FIXa antibodies secreted by hybridoma cells, the commercially available test-kit COATEST VIII:C/4® (Chromogenix) was employed. The assay was done essentially as described by the manufacturer with the following modifications:

To allow high throughput screening, the assay was down-scaled to microtiter plate format. Briefly, 25 µl aliquots of hybridoma supernatants were transferred to microtiter plate (Costar, #3598) wells and warmed to 37° C. Chromogenic substrate (S-2222), synthetic thrombin inhibitor (1-2581), factor (F) IXa and FX were reconstituted in sterile water and FIXa/FX was mixed with phospholipids according to the supplier's protocol. Per reaction, 50 µl of the phospholipid/FIXa/FX solution were combined with 25 µl CaCl<sub>2</sub> (25 mM) and 50 µl of the substrate/inhibitor cocktail. To start the reaction, 125 µl of the premix were added to the hybridoma supernatant in the microtiter plates and incubated at 37° C. Absorbency at 405 nm and 490 nm of the samples was read at various times (30 min to 12 h) against a reagent blank (MLW, cell culture medium instead of hybridoma supernatant) in a Labsystems iEMS Reader MF™ microtiter plate reader. FVIII-like activity of the samples was calculated by comparing the absorbency of the samples against the absorbency of a diluted FVIII reference standard (IMMUNO AG # 5T4AR00) using GENESIS™ software.



US 7,033,590 B1

11

The results of a screening for FVIII-like activity in hybridoma cell culture supernatants are shown in FIG. 1. Pre-selected clones derived from fusion experiments #193, #195 and #196 (see above) were examined in a chromogenic FVIII assay as described. Clones 193/M1, 193/N1 and 193/P1 are subclones derived from the master clone 193/C0 (see below). Master clone 195/10 was derived from fusion experiment #195 and clones 196/A0, 196/B0 and 196/C0 were derived from fusion experiment #196. In a typical screening experiment, approximately 1000 clones (in 96 wells) from a single fusion experiment were pre-screened for FVIII-like activity. Subsequently, selected clones were grown on a larger scale (3–5 ml supernatant) and re-analyzed in a chromogenic assay. As a negative control cell culture medium was assayed on each plate (MLW).

Wells either exhibiting high FVIII-like activity or substantial FVIII-like activity were subjected to subcloning procedures. The selection and subcloning process is exemplified for the screening and subcloning of an IgG producing cell line (i.e. 193/C0) but has been done exactly the same way for an IgM (i.e. 196/C0, see below, FIG. 5) producing clone.

The selection process was done by initially plating all hybridoma cell clones derived from a single fusion experiment on ten 96 well plates thereby creating the so called "master plates". Singular positions (wells) on a master plate usually contained more than one hybridoma cell clone (usually 3 to 15 different clones). Subsequently, the antibody secreted by only several thousand cells was tested. These cells grew under conditions suboptimal for antibody production, which is known to be best in dying cells. So the expected specific anti-FIX antibody concentration in the supernatant may be in the range of 10–12 to 10–14 M. This explains why incubation periods had to be extended compared to standard FVIII assays.

Results of a screening for an IgG mediated FVIII-like activity in hybridoma cell culture supernatants of a master plate are shown in FIG. 2. Supernatants were examined in a chromogenic FVIII assay. Shown are the results derived from the fifth master plate of fusion experiment number #193 (Balb/c mice immunized with FIX $\alpha$ ). Absorbance was read after 4 hours of incubation at 37° C. Position ES was identified as exhibiting FVIII like activity significantly higher than the blank (MLW). This cell pool was designated 193/C0 and was further subcloned (FIG. 3). As each well of the master plate contains more than one hybridoma cell clone, cells of a single positive well were expanded and plated at a calculated cell density of 2–0.2 cells/well on a 96 well plate. Again, the supernatants were tested for FVIII-like activity and positive positions were subjected to another round of subcloning. Typically three to four rounds of subcloning were performed with each clone displaying FVIII-like activity to obtain homogenous cell populations. Here the results of the chromogenic assay of the 193/C0 subclones are shown. Absorbance was read after a 4 hour incubation period at 37° C. Positions A6 and D5 exhibited substantial FVIII-like activity and were named 193/M1 and 193/P1, respectively. These two clones were subjected to another round of subcloning. As a negative control plain cell culture medium was assayed on each plate (MLW(H1)).

A comparison of chromogenic FVIII-like activity and FIX-ELISA reactivity of small scale (3 ml) hybridoma cultures is shown in FIG. 4. Before a decision was made whether a master clone (or subclone) was to be further subcloned, clones were grown at a 3–5 ml scale and the supernatants were checked again. This graph shows the FIX specific ELISA results and the FVIII-like chromogenic

12

activity of the master clone 193/C0 and all its subclones which were identified as positives and re-checked. Blanks (absorbency of the chromogenic reagent itself) were subtracted in the case of the ELISA as well as the chromogenic assay readings depicted here. Clone 193/M1 was subcloned and yielded clones 193/V2, 193/M2 and 193/U2. The other clones of the 2<sup>nd</sup> round came from 193/P1, 193/AB2 and 193/P2 were subcloned. 193/AF3, 193/AB3 and 193/AE3 are subclones of 193/AB2. The other clones of the 3<sup>rd</sup> round came from 193/P2. Finally 193/AF3 ( $\rightarrow$ 193/AF4), AE3 ( $\rightarrow$ 193/AE4, 193/AL4, 193/AN4 and 193/AO4) and 193/AD3 ( $\rightarrow$ 193/AG4, 193/AH4, 193/AD4, 193/AI4, 193/AK4) were subcloned.

From each fusion experiment, several (5–15) master clones (selected from the master plate) were identified and subjected to subcloning. After 3 rounds of sub-cloning, most of the cell lines were homogenous as demonstrated by ELISA and chromogenic activity analysis (see FIG. 4) as well as by cDNA sequence analysis. A specific master clone and all its subclones produce the same FIX/FIX $\alpha$  binding antibody. However, there are huge differences in the antibody protein sequences of clones derived from different master clones (see Example 11). Most hybridoma cell lines express antibodies from the IgG subclass (i.e. clones #193, #198, like 198/A1, 198/B1, 198/BB1). However, we were also able to select some clones expressing IgM antibodies.

The chromogenic activity of hybridoma supernatant of some important master clones and subclones was determined. Absorbance was measured after a 1 h 30 min and 3 h 30 min incubation period at 37° C. (FIG. 5). In contrast to all the clones from the 193<sup>rd</sup> fusion, clone 196/C0 and its subclone 196/AP2 produced a FIX/FIX $\alpha$ -specific IgM antibody that gave a strong chromogenic activity even after a short period of incubation.

The following cell lines have been deposited with the European Collection of Cell Cultures (ECACC) in accordance with the Budapest Treaty: 198/B1 (ECACC No. 99090925, deposited Sep. 9, 1999); 198/A1 (ECACC No. 99090924, deposited Sep. 9, 1999); 198/BB1 (ECACC No. 99090926, deposited Dec. 16, 1999); 193/AO (ECACC No. 99121614, deposited Dec. 16, 1999); 196/C4 (ECACC No. 99121615, deposited Dec. 16, 1999); 198/DI (ECACC No. 99121616, deposited Dec. 16, 1999); 198/T2 (ECACC No. 99121617, deposited Dec. 16, 1999); 198/G2 (ECACC No. 99121618, deposited Dec. 16, 1999); 198/AC1 (ECACC No. 99121619, deposited Dec. 16, 1999); and 198/U2 (ECACC No. 99121620, deposited Dec. 16, 1999). The address of the ECACC is Salisbury, Wiltshire SP4 0JG, UK.

To do a more in depth analysis of the biochemical properties of certain antibodies, homogenous hybridoma cell lines expressing different antibodies with FVIII-like activity were expanded and used to express the antibody in question on a larger scale (100–1000 ml). These antibodies were affinity purified (see Example 3) prior to being used in further experiments.

### Example 3

#### Factor IX/FIX $\alpha_{(\alpha,\beta)}$ Binding Properties of Antibodies Exhibiting FIX/FIX $\alpha$ Activating Activity

Factor IX and the two activated forms of FIX, FIX $\alpha$  and FIX $\alpha\beta$  (FIX/FIX $\alpha_{(\alpha,\beta)}$ ) were diluted in TBS (25 mM Tris HCl, 150 mM NaCl, pH 7.5) to a final concentration of 2  $\mu$ g/ml. Nunc Maxisorp ELISA plates were coated with 100  $\mu$ l FIX/FIX $\alpha_{(\alpha,\beta)}$  solution according to standard procedures (4° C., overnight) and washed several times with TBST

US 7,033,590 B1

13

(TBS, 0.1% (v/v) Tween 20). 50  $\mu$ l hybridoma supernatant was diluted 1:1 with 50  $\mu$ l TBST/2% BSA and added to the coated ELISA plate. After an incubation period of 2 h at room temperature (RT), plates were washed 4 times with TBST and incubated (2 h, RT) with 100  $\mu$ l/well of a 1:25000 dilution (in TBST/1% BSA) of an anti-mouse IgG (Fc-specific) peroxidase conjugated antibody (Sigma, #A-0168). Wells were washed 5 times with TBST and finally stained with 100  $\mu$ l freshly prepared staining solution (10 ml 50M sodium citrate, pH 5 supplemented with 100  $\mu$ l OPD (60 mg OPD/ml) and 10  $\mu$ l 30%  $H_2O_2$ ). The reaction was stopped by the addition of 50 ml  $H_2SO_4$  and the optical density recorded at 492 nm and 620 nm in a Labsystems iEMS Reader MF<sup>TM</sup> microtiter plate reader employing GENESIS<sup>TM</sup> software.

In certain cases, instead of an anti-mouse IgG ELISA, an anti-mouse IgM ELISA was carried out.

Purification of Mouse-IgG from Hybridoma Cell Culture Supernatants

Hybridoma supernatant (100–500 ml) was supplemented with 200 mM Tris/HCl buffer (pH 7.0) and solid NaCl to give final concentrations of 20 mM Tris and 3M NaCl, respectively. The supernatant was then clarified by centrifugation at 5500xg for 10 minutes. A 1 ml protein G affinity chromatography column (Protein G Sepharose Fast Flow, Amersham-Pharmacia) was washed with 15 ml 20 mM Tris/Cl pH 7.0 and afterwards equilibrated with 10 ml of 20 mM Tris/Cl buffer pH 7.0 containing 3M NaCl. The hybridoma supernatant containing 3M NaCl was then loaded onto the column by gravity. The column was washed with 15 ml of 20 mM Tris/Cl buffer, pH 7.0, containing 3M NaCl. Bound IgG was further eluted with 12 ml glycine/HCl buffer pH 2.8 and 1 ml fractions were collected. 100  $\mu$ l of 1M Tris pH 9.0 were added to each fraction for neutralization. Fractions containing the IgG were identified by mixing 50:1 with 150  $\mu$ l of a staining solution (BioRad concentrate, 1:5 diluted with water) in wells of a microplate. Positive fractions were pooled, concentrated to 1 ml in an ultrafiltration concentrator device (Centricon Plus 20, Amicon) according to the manufacturer. The concentrate was diluted with 19 ml TBS (20 mM Tris/Cl buffer pH 7.0 containing 150 mM NaCl) and again concentrated to 1 ml. The diluting-concentrating step was repeated for two more times in order to bring IgG into TBS.

Purification of Mouse-IgM from Hybridoma Cell Supernatants

100–500 ml of hybridoma cell culture supernatant were concentrated to 5–10 ml either with an ultra-filtration concentrator device (Centricon Plus 20, Amicon) according to the manufacturer or by ammonium sulfate precipitation (40% saturation, 0° C.) and redissolving the precipitate with 5–10 ml of TBS. In either case the concentrate was dialyzed against 20 mM Tris Cl buffer pH 7.4 containing 1.25M NaCl and further concentrated to 1 ml in a Centricon Plus 20, (Amicon) ultrafiltration device. IgM was purified from this concentrate with the Immunopure IgM Purification Kit (Pierce) according to the manufacturer. Fractions collected during elution from the maltose binding protein-column were tested for IgM, pooled, concentrated and brought into TBS as described for IgG.

Determination of IgG Concentrations in Purified Preparations

Total IgG content 280 nm–extinction of appropriate dilutions were measured. E280=1.4 corresponds to 1 mg/ml protein.

Factor IXa Specific IgG (Quantitative ELISA)

Wells of a microplate (Nunc Maxisorp) were incubated with 2  $\mu$ g/ml factor IXa diluted in TBS (25 mM Tris/HCl pH

14

7.5 containing 150 mM NaCl) overnight at 4° C. Wells were washed four times with TBST (25 mM Tris/HCl pH 7.5 containing 150 mM NaCl and 0.1% (v/v) Tween 20). As a standard monoclonal AB the HIX1 anti-FIX (accurate) was used. Standard and samples were diluted in TBST containing 2%(w/v) BSA. The standard dilution series and appropriate dilutions of the samples were incubated on the ELISA-plate for 2 hours at room temperature. Plates were washed 4 times with TBST and incubated (2 h, RT) with 100  $\mu$ l/well of a 1:25000 dilution (in TBST/1% BSA) of an anti-mouse IgG (Fc-specific) peroxidase conjugated antibody (Sigma, #A-0168) FIXa. Wells were washed 5 times with TBST and finally stained with 100  $\mu$ l freshly prepared staining solution (10 ml 50 mM sodium citrate, pH 5 supplemented with 100  $\mu$ l OPD (60 mg OPD/ml) and 10  $\mu$ l 30%  $H_2O_2$ ). The reaction was stopped by the addition of 50 ml  $H_2SO_4$  and after 30 minutes the optical density was recorded at 492 nm and 620 nm in a Labsystems iEMS Reader MF<sup>TM</sup> microtiter plate reader employing GENESIS<sup>TM</sup> software.

#### Example 4

##### Anti-FIX/FIXa Antibodies Exhibiting FVIII-like Activity in a Chromogenic FVIII Assay

Several anti-FIX/FIXa antibody producing hybridoma clones were subcloned up to four times and the resulting monoclonal hybridoma cell line used to produce monoclonal antibody containing supernatant. IgG isotype antibodies derived from these supernatants were purified over affinity columns and dialyzed against TBS (see above). IgM antibodies were used as unpurified supernatant fractions. The following experiments were done with two sets of representative antibodies: 193/AD3 and 198/AC1/1 (IgG isotype, the antibody 198/AC1/1 is a preparation from the parent 198/AC1 hybridoma clone, i.e. that a (frozen) vial containing 198/AC1 cells is cultivated and antibodies are produced. The supernatant is then used for these experiments.) and 196/AF2 and 196/AF1 (IgM isotype) (FIG. 6A and FIG. 6B). Briefly, 25  $\mu$ l aliquots of monoclonal antibody containing sample (unpurified hybridoma supernatant or, where indicated, a certain amount of FIX specific antibody) were transferred to microtiter plate wells and warmed to 37° C. Chromogenic substrate (S-2222), synthetic thrombin inhibitor (I-2581), factor (F) IXa and FX were reconstituted in sterile water and FIXa/FX was mixed with phospholipids according to the supplier's protocol. Per reaction, 50  $\mu$ l of the phospholipid/FIXa/FX solution were combined with 25  $\mu$ l  $CaCl_2$  (25 mM) and 50  $\mu$ l of the substrate/inhibitor cocktail. To start the reaction, 125  $\mu$ l of the premix were added to the monoclonal antibody solution in the microtiter plates and incubated at 37° C. Absorbance at 405 nm and 490 nm of the samples was read at various times (5 min to 6 h) against a reagent blank (cell culture medium instead of hybridoma supernatant) in a Labsystems iEMS Reader MF<sup>TM</sup> microtiter plate reader using GENESIS<sup>TM</sup> software.

The time course of FVIII-like activity exhibited by monoclonal antibodies 193/AD3 (IgG isotype) and 196/AF2 (IgM isotype) compared to human FVIII (12 and 16 mU/ml), TBS and to cell culture medium is shown in FIG. 6A. After a lag phase, both antibodies give rise to chromogenic substrate cleavage, as judged by the increasing optical density measurable at 405 nm wavelength.

The time course of FVIII-like activity exhibited by monoclonal antibodies 198/AC1/1 (IgG isotype, 10  $\mu$ g/ml) and 196/AF1 (IgM isotype, unpurified supernatant) compared to human FVIII (16 mU/ml) and 10  $\mu$ g/ml of mouse IgG is

US 7,033,590 B1

15

shown in FIG. 6B. After a lag phase, both antibodies give rise to chromogenic substrate cleavage, as judged by the increasing optical density measurable at 405 nm wavelength.

#### Example 5

##### FVIII-like Activity Exhibited by Anti-FIX/FIXa-antibodies Generates Factor Xa and is Phospholipid, FIXa/FX and $\text{Ca}^{2+}$ Dependent

Factor VIII activity is usually determined with a chromogenic assay and/or an APTT-based clotting assay. Both types of assays rely on FVIIIa/FIXa-mediated factor Xa generation. In the case of a chromogenic FVIII assay, the factor Xa produced will subsequently react with a chromogenic substrate, which can be monitored spectroscopically, e.g., in an ELISA reader. In an APTT based clotting assay free factor Xa will assemble with FVa on a phospholipid surface in the so-called prothrombinase complex and activate prothrombin to thrombin. Thrombin in turn gives rise to fibrin generation and finally to clot formation. Central to the two assay systems is generation of factor Xa by the FVIIIa/FIXa complex.

To demonstrate that the FVIII-like activity exhibited by anti-FIX/FIXa-antibodies indeed generates factor Xa, the following experiment was carried out. Several 25  $\mu\text{l}$  aliquots of unpurified hybridoma supernatant 196/AF2 (IgM isotype) were transferred to microtiter plate wells and warmed to 37° C. As a positive control, 16mU of Recombinate™ were diluted into hybridoma medium (196 HM 007/99) and treated exactly the same way as the hybridoma supernatant. As a negative control, plain hybridoma medium was used. Chromogenic substrate (S-2222), synthetic thrombin inhibitor (I-2581), factor IXa and FX were reconstituted in sterile water and FIXa/FX was mixed with phospholipids according to the supplier's protocol. Pefabloc Xa®, a factor Xa specific proteinase inhibitor (Pentapharm, LTD), was reconstituted with water to a final concentration of 1 mM/l. Per reaction, 5011 of the phospholipid/FIXa/FX solution were combined with 25  $\mu\text{l}$   $\text{CaCl}_2$  (25 mM) and 50  $\mu\text{l}$  of the substrate/thrombin-inhibitor cocktail. To start the reaction, 125  $\mu\text{l}$  of the premix were added to the samples in the microtiter plates and incubated at 37° C. Where indicated, 35  $\mu\text{M}$  Pefabloc Xa® were added. Absorbance at 405 nm and 490 nm was read at various times (every 5 minutes to 6 h) against a reagent blank (cell culture medium) in a Lab-systems iEMS Reader MF™ microtiter plate reader employing the GENESIS™ software.

The results of the factor IXa stimulation by the FVIII-like activity exhibited by the IgM anti FIX/FIXa-antibody 196/AF2 in generating factor Xa as judged by the readily measurable cleavage of the chromogenic substrate S-2222 (compare "16 mU FVIII" and "196/AF2") is shown in FIG. 7A. Factor Xa activity is effectively blocked by the FXa specific inhibitor "Pefabloc Xa®" (compare "196/AF2" versus "196/AF2 351M Pefabloc Xa®") indicating that indeed FXa was generated.

The same experiment was performed using purified IgG preparations of clone 198/AM1 (FIG. 7B). Purified IgG was diluted in TBS to a final concentration of 0.4 mg/ml and 25  $\mu\text{l}$  (i.e. a total of 10  $\mu\text{g}$ ), transferred to microtiter plate wells and warmed to 37° C. As a positive control, 6 mU plasma derived FVIII was used. 10  $\mu\text{g}$  unspecific mouse IgG (Sigma, I-5381) served as a negative control. The assay was performed as described above.

Further experiments show the factor IXa stimulation by the FVIII-like activity exhibited by the IgG anti-FIX/FIXa-

16

antibody 198/AM1 generates factor Xa as judged by the readily measurable cleavage of the chromogenic substrate S-2222 (FIG. 7B). Again factor VIII and antibody 198/AM1 generate FXa which is effectively blocked by the FXa specific inhibitor "Pefabloc Xa®". As a negative control, unspecific mouse IgG (Sigma, 15381) was assayed.

In another set of experiments, the dependence of the FVIII-like activity of either purified anti-FIX/FIXa-antibodies (IgM, FIG. 8A) or of unpurified antibodies derived from cell culture supernatants (IgG, FIG. 8B) on the presence of phospholipids (PL), FIXa/FX and  $\text{Ca}^{2+}$  was demonstrated. Mouse IgG was used as a control (FIG. 8C). Factor VIII-like activity was assayed essentially as described above. When indicated, either the FIXa/FX mixture, the PL or  $\text{Ca}^{2+}$  was omitted from the reaction. Absorbency at 405 nm and 490 nm of the samples was read at various times against a reagent blank (buffer instead of purified antibody) in a Labsystems iEMS Reader MF™ microtiter plate reader. The results are shown in FIG. 8A, FIG. 8B and FIG. 8C.

The dependence of the FVIII-like activity of purified anti-FIXa-antibody 198/AC1/1 (IgG isotype, concentration used throughout the assay was 10  $\mu\text{g}/\text{ml}$ ) on the presence of phospholipids (PL), FIXa/FX and  $\text{Ca}^{2+}$  is further shown in FIG. 8A. As is easily recognizable, only the complete assay, including antibody, PL,  $\text{Ca}^{2+}$ , and FIXa/FX gives rise to a reasonable FXa generation. The dependence of the FVIII-like activity of cell culture supernatant containing unpurified IgM isotype anti-FIX/FIXa-antibody (196/AF1) on the presence of phospholipids, FIXa/FX and  $\text{Ca}^{2+}$  is shown in FIG. 8B.

Again, as already shown for the purified IgG preparation (FIG. 8A), antibody 198/AC1/1, only the complete assay, including PL,  $\text{Ca}^{2+}$ , FIXa/FX, will give a reasonable amount of FXa generation. To demonstrate the specificity of the reaction, total IgG prepared from normal mouse plasma was assayed under the same conditions as above. The results are shown in FIG. 8C. No FVIII-like activity could be detected. There is, as expected, no activity detectable in the absence of phospholipids, FIXa/FX and  $\text{Ca}^{2+}$ . All experiments were done in a microtiter plate and the OD405 was scanned every 5 minutes for 6 h.

#### Example 6

##### Certain anti-FIX/FIXa-antibodies are procoagulant in the presence of FIXa

During normal hemostasis, FIX becomes initially activated either by the tissue factor (TF)/factor VIIa pathway or later on by activated factor XI (FXIa). Subsequent to its activation, FIXa associates on the platelet surface in a membrane bound complex with activated FVIII. Factor IXa by itself has little or no enzymatic activity towards FX, but becomes highly active in the presence of FVIIIa. To demonstrate that certain anti-FIX/FIXa antibodies have FVIII-like activity and hence are procoagulant in a FVIII deficient human plasma, the following experiment was carried out. Different amounts of antibody 193/AD3 or mouse IgG (as a control) were used in a standard aPTT based one stage clotting assay. Briefly, 100  $\mu\text{l}$  of antibody-containing samples were incubated with 100  $\mu\text{l}$  of FVIII deficient plasma (DP) and with 100  $\mu\text{l}$  of DAPTTIN (PTT Reagent for determining activated Thromboplastin Time; IMMUNO AG) reagent, in a KC10A clotting analyzer. Where indicated, a total amount of 50 ng activated FIX was included in the reaction mixture. After a 4 minute

US 7,033,590 B1

17

incubation, the reaction was started by the addition of 100  $\mu$ l  $\text{CaCl}_2$  (25 mM). The results are shown in Table 1 and FIG. 9.

$\mu$ g AB	clotting time (sec)	
	193/AD3 50 ng FIXa	mouse IgG 50 ngFIXa
9	101.6	102.5
4.5	95.6	103.2
2.25	93.1	103.2
1.8	93.7	101.9
1.35	91.4	103.4
0.9	94.4	102.2
0.45	98.1	101.9
0.34	97.1	103.9
0.23	99.3	103.7

Table 1: Clotting times of FVIII deficient plasma in an APTT based clotting assay employing various amounts of procoagulant (193/AD3) and control antibody (mouse IgG) in the presence of 50 ng activated FIX (0.01UFIX). The molar ratio of antibody in the reaction and activated FIX is 10:1. The molar ratio between antibody and total FIX (FIX and FIXa, assuming that human FVIII deficient plasma contains 1 U (5% g) FIX) varies between 6:1 (9% g antibody in reaction) and 1:6 (0.23% g antibody in reaction). At the optimal shortening of the clotting time, the molar ratio between antibody and total FIX is 1:1. The clotting time without the addition of FIXa is in the range of 120 seconds.

FIG. 9 is a graphical representation of the clotting times of FVIII deficient plasma in an aPTT based clotting assay employing various amounts of procoagulant (193/AD3) and control (mouse IgG) antibody in the presence of 50 ng activated FIX. There is a clear dose-dependent reduction of the clotting time in samples supplemented with antibody 193/AD3. These results imply that antibody 193/AD3 is procoagulant in the presence of FIXa.

#### Example 7

##### Anti-FIX/FIXa-antibodies are Procoagulant in the Presence of FVIII Inhibitors and FIXa

A severe complication of the standard FVIII substitution therapy is the development of alloantibodies directed against FVIII, leading to FVIII neutralization and a condition where the patient's blood will not clot.

To demonstrate that certain anti-FIXa-antibodies have FVIII-like activity even in the presence of FVIII inhibitors, the following experiment was carried out. Different amounts of antibody 193/AD3 or, as a control, mouse IgG were used in a standard APTT based one-stage clotting assay. Briefly, 100  $\mu$ l antibody samples were incubated with either 100  $\mu$ l of FVIII deficient plasma (FIG. 10A) or FVIII inhibitor plasma (inhibitor potency 400 BU/ml), FIG. 10B) as well as with 100  $\mu$ l of DAPTTIN reagent, in a KC10A clotting analyzer. In addition, a total amount of 50 ng activated FIXa was included in the reaction mixture. After a 4 minute incubation, the reaction was started by the addition of 100  $\mu$ l  $\text{CaCl}_2$  (25 mM). To ensure equal conditions, the experiments employing FVIII deficient plasma and FVIII inhibitor plasma were done side by side. The results are shown in FIGS. 10A and 10B. As already shown in Example 6, there is a clear dose-dependent reduction of the clotting time in samples supplemented with antibody 193/AD3 in the presence of FVIII inhibitors.

18

#### Example 8

##### Anti-FIX/FIXa-antibodies are Procoagulant in the Presence of Defective FVIII and FIXa

To demonstrate that certain anti-FIXa-antibodies have FVIII-like activity in the presence of defective FVIII, the following experiment may be carried out. Increasing amounts of antibody 193/AD3 or, as a control, mouse IgG are used in a standard aPTT-based one stage clotting assay. In this clotting assay, a hemophilia A patient's plasma having very low clotting activity due to the presence of defective FVIII (DF8) is used. Briefly, 100  $\mu$ l antibody samples are incubated with either 100  $\mu$ l of DF8 plasma or FVIII deficient plasma as well as with 100  $\mu$ l of DAPTTIN reagent, in a KC10A clotting analyzer. In addition, a total amount of 50 ng activated FIXa is included in the reaction mixture. After a short incubation, the reaction will be started by the addition of 100  $\mu$ l  $\text{CaCl}_2$  (25 mM). To ensure equal conditions, the experiment employing FVIII deficient plasma and DF8 plasma is done side by side.

#### Example 9

##### Anti-FIX/FIXa-antibodies with Procoagulant Activity in the Presence of FIXa Distinguish Between Human and Bovine FIXa

FIX/FIXa specific monoclonal antibodies selected from the 198<sup>th</sup> fusion experiment were purified from the respective hybridoma supernatant and quantified as described in Example 3. These antibodies were analyzed in a modified one-stage clotting assay (as described in Example 6) and some showed procoagulant activity.

The chromogenic activity of these antibody preparations was measured in the following FIXa generation kinetic assay: 10  $\mu$ g of monoclonal antibody (in 25  $\mu$ l) were transferred to microtiter plate wells and warmed to 37° C. Chromogenic substrate (S-2222), synthetic thrombin inhibitor (I-2581), factor IXa and FX were reconstituted in sterile water and FIXa/FX (both bovine) were mixed with phospholipids according to the supplier's protocol. Per reaction, 50  $\mu$ l of the phospholipid/FIXa/FX solution were combined with 25  $\mu$ l  $\text{CaCl}_2$  (25 mM) and 50  $\mu$ l of the substrate/inhibitor cocktail. To start the reaction, 125  $\mu$ l of the premix were added to the monoclonal antibody solution in the microtiter plates and incubated at 37° C. Absorbance at 405 nm and 490 nm of the samples was read at various times (5 min to 2 h) against a reagent blank (25 ml TBS instead of monoclonal antibodies) in a Labsystems iEMS Reader MFT<sup>TM</sup> microtiter plate reader using GENESIS<sup>TM</sup> software. In parallel, the same reactions were performed except that 50 ng human FIXa were added per reaction. Those antibodies that showed procoagulant activity had no chromogenic activity in the case of bovine FIX, but displayed high activity when human FIXa was present.

FIG. 11 shows the time course of the FVIII-like activity exhibited by the monoclonal antibodies 198/A1, 198/B1 and 198/AP1 with (+) and without (-) addition of 50 ng human FIXa $\beta$ . Non-specific polyclonal mouse IgG was used as a control. 198/A1 and 198/B1 show procoagulant activity (similar as 193/AD3 in example 6) whereas 198/AP1 does not. Antibody 198/BB1 had the same activity pattern (data not shown).

Further monoclonal antibodies selected from the 198<sup>th</sup> fusion experiment include 198/DI (ECACC No. 99121616), 198/T2 (ECACC No. 99121617), 198/G2 (ECACC No.9912118), 198/U2 (ECACC No. 99121620).

US 7,033,590 B1

19

## Example 10

Structure and Procoagulant Activity of Antibody  
Derivatives Derived from Anti-FIX/FIXa-  
antibodies; Subcloning Antibody Variable Domains  
from Hybridoma Cell Lines 193/AD3, 193/K2,  
198/A1 and 198/B1 (Clone AB2)

Cloning procedure: Messenger RNA was prepared from  $1 \times 10^6$  hybridoma cells of the respective cell line (either 193/AD3, 193/K2, 198/A1 or 198/B1 (clone AB2)) employing the "QuickPrep® Micro mRNA Purification Kit" (Pharmacia) according to the manufacturer's instructions. The corresponding cDNA was produced by retro transcription of mRNA using the "Ready-To-Go-You-Prime-First-Strand Beads kit" (Pharmacia) according to the manufacturer's instructions. Heavy and light chain encoding sequences were converted to the corresponding cDNA employing a set of primers. To reverse transcribe heavy chain-specific mRNA (VH), an equimolar mixture of the oligonucleotides MOCG1-2FOR (5' CTC AAT TTT CTT GTC CAC CTT GGT GC 3') (SEQ.ID.NO. 1), MOCG3FOR (5' CTC GAT TCT CTT GAT CAA CTC AGT CT 3') (SEQ.ID.NO. 2) and MOCMFOR (5' TGG AAT GGG CAC ATG CAG ATC TCT 3') (SEQ.ID.NO. 3) was used (RTmix1). In the same reaction tube, light chain-specific cDNA (VL) was synthesized using primer MOCKFOR- (5' CTC ATT CCT GTT GAA GCT CTT GAC 3') (SEQ.ID.NO. 4).

The coding sequences for VH were amplified by PCR using the primer-sets depicted in FIG. 12 and the specific cDNA, derived from the reverse transcription mixture (RTmix1) described above, as the template. VK-chain genes were amplified using the primer sets depicted in FIG. 13 and also employing RTmix1 as a template. The VF-PCR product was cleaved SfiI-AscI and inserted into SfiI-AscI digested vector pDAP2 (GeneBank accession no.: U35316). The pDAP2-VH constructs obtained thereby were named pDAP2-193AD3/VH, pDAP2-198A1/VH, pDAP2-198AB2/VH (derived from antibody 198/B1) and pDAP2-193K2/VH, respectively. The plasmids were subsequently cleaved with AscI-NotI and the corresponding AscI-NotI digested VK-gene PCR product was inserted. The resultant vectors were designated pDAP2-193/AD3scFv, pDAP2-198/A1scFv, pDAP2-198/AB2scFv (derived from antibody 198/B1) and pDAP2-193/K2scFv and code for the VH-gene and the VL-gene of the monoclonal antibodies 193/AD3, 198/A1, 198/AB2 (derived from antibody 198/B1) and 193/K2. Heavy and light chains are linked by the coding sequence for an artificial, flexible linker ( $G_4SGGRASG_4S$  (SEQ ID NO:111); Engelhardt et al., 1994) and enables expression of the scFv variant of the respective antibody.

In FIG. 14, the DNA and the deduced protein sequence of the scFv derived from the hybridoma cell line 193/AD3 are depicted. Nucleotides 1 to 357 code for the heavy chain variable domain, nucleotides 358 to 402 code for the artificial flexible linker and nucleotides 403 to 726 code for the light chain variable region. The protein sequence of the CDR3 region of the heavy chain has the sequence YGNSP-KGFAY (SEQ ID NO:5) and is given in bold letters. The artificial linker sequence ( $G_4SGGRASG_4S$ ; SEQ ID NO:111) is shown.

In FIG. 15, the DNA and the deduced protein sequence of the scFv derived from the hybridoma cell line 193/K2 is shown. Nucleotides 1 to 363 code for the heavy chain variable domain, nucleotides 364 to 408 code for the artificial flexible linker, and nucleotides 409 to 747 code for the

20

light chain variable region. The protein sequence of the CDR3 of the heavy chain has the sequence DGGHGYGSS-FDY (SEQ ID NO:6), and is given in bold letters. The artificial linker sequence ( $G_4SGGRASG_4S$ ; SEQ ID NO:111) is shown.

In FIG. 16, the DNA and the deduced protein sequence of the scFv derived from the hybridoma cell line 198/AB2 (derived from antibody 198/B1) are depicted. Nucleotides 1 to 366 code for the heavy chain variable domain, nucleotides 367 to 411 code for the artificial flexible linker, and nucleotides 412-747 code for the light chain variable region. The protein sequence of the CDR3 region of the heavy chain has the sequence EGGGFTVNWYFDV (SEQ ID NO:7) and is given in bold letters. The artificial linker sequence ( $G_4SGGRASG_4S$ ; SEQ ID NO:111) is also shown.

In FIG. 17, the DNA and the deduced protein sequence of the scFv derived from the hybridoma cell line 198/A1 are depicted. Nucleotides 1 to 366 code for the heavy chain variable domain, nucleotides 367 to 411 code for an artificial flexible linker, and nucleotides 412-747 code for the light chain variable region. The protein sequence of the CDR3 region of the heavy chain has the sequence EGGGYVNWYFDV (SEQ ID NO:8) and is given in bold letters. The artificial linker sequence ( $G_4SGGRASG_4S$ ; SEQ ID NO:111) is also shown.

## Example 11

Procoagulant Activity of Peptides Derived from  
CDR3 Regions of Anti-FIX/FIXa-Antibodies

In principle, the antibody molecule can be envisioned as a biological device for the presentation of a combinatorial array of peptide elements in three dimensional space (see Gao et al., 1999, PNAS, 96:6025). Therefore, an antibody (or an antibody derivative, e.g. scFv, Fab, etc.) can be used either as a tool for the detection of functionally important domains of a specific target protein, or on the other hand, for the delineation of amino acid sequences specifically mediating certain interactions, i.e. activating or enhancing the activity of FIXa towards the physiological substrate FX. The latter process has led to the evaluation of a number of heavy chain CDR3 region (CDR3H) derived peptide sequences as FIXa enhancing agents.

Enhancing the procoagulant activity of peptides which exhibit such activity may be accomplished through sequence variation within the peptide regions critical for mediating the FIXa activity enhancement. As a possible step towards peptide sequences with enhanced procoagulant activity, the binding site of an antibody, i.e. 198/A1 or 198/B1, on the FIXa molecule is mapped by employing sequence comparison analyses, competitive binding assays, Western blot analyses and competitive ELISA analyses. Since the crystal structure of FIX is known, molecular modeling is subsequently used to improve the fitting of i.e. 198/B1 derived peptides in the 198/B1 binding site on human FIXa.

On the other hand, methodical mutational analysis of a given peptide sequence such as 198/A1 or 198/B1 CDR3H derived peptide sequences by, e.g., "alanine scanning mutational analysis" allows for the identification of peptide residues critical for procoagulant activity. Another way to improve the activity of a certain peptide sequence is the use of peptide libraries combined with high throughput screening.

The antigen binding site of an antibody is derived from the juxtaposition of the six "complement determining regions (CDR's)" at the N-terminal end of the VL-HL dimer

US 7,033,590 B1

21

(or Fv region). The contribution of a single CDR to the antibody specificity for a given antigen may vary considerably, but in general it is thought that the CDR3 region of the heavy chain (CDR3H) is of special influence, i.e. the particular protein sequence of CDR3<sub>H</sub> region may be highly important for antigen recognition. The length of CDR3H regions has been reported to vary considerably and is in the range of 4–25 amino acids (Borrebaeck, p. 16).

An example of a methodical mutational analysis of peptide sequences is given below. To improve the solubility/procoagulant efficacy of peptides derived from the CDR3H region of anti FIX/FIXa antibodies, the N-terminal as well as the C-terminal amino acid sequences were changed. In addition, a series of mutated peptides was constructed and analyzed.

The principle of such a study is exemplified by a series of peptides derived from CDR3H region of antibodies 198/A1 and 198/B1. The original peptide A1 (see table 2) is derived from the CDR3H region of antibody 198/A1 and peptide B1 is derived from the CDR3H region of antibody 198/B1, respectively (see example 10, FIGS. 16 and 17). The term “scrambled version” means that a peptide has the same amino acids but in random order.

22

FVIII assay was developed (see examples 2 and 4). The basic principle is, that without a cofactor, FIXa will have very limited activity towards its natural substrate FX. Only in the presence of a substance having FIXa activation properties, i.e. FVIII or a substance exhibiting FVIII-like activity, a substantial amount of FXa is produced by cleavage of FX through the FIXa/activator complex. The amount of FXa generated is monitored by cleavage of a chromogenic substrate. The principle of the revised chromogenic assay is described for two representative peptides: A1/3 and A1/5 (Table 2). Briefly, 25 µl aliquots of peptide stock solution (in imidazole buffer (IZ) 50 mM imidazole, 100 mM NaCl, pH7.2) were transferred to microtiter plate wells and warmed to 37° C. Chromogenic FXa substrate (S-2222), synthetic thrombin inhibitor (I-2581), bovine FIXa and bovine FX were reconstituted in sterile water and FIXa/FX mixed with phospholipids according to the supplier's protocol. Since the peptides do not react with bovine FIXa, (which comes as a mixture with bovine FX in the Test Kit) 2.9 nM (in most cases 2.3 nM) human FIXa (ERL) were added (see Example 11, FIG. 19). Per reaction, 50 µl of the phospholipid/FIXa/FX solution were combined with 251 CaCl<sub>2</sub> (25 mM) and 50 µl of the substrate/inhibitor cocktail. To start the reaction, 125 µl of the premix were added to the

Peptide	Sequence	Amino-acids	MW (D)	pI	Remark
A1	EGGGYYVNWYFDV (SEQ ID NO:8)	(13aa)	1569	7.2	Decreased solubility
A1/1	VYGFGWGYEVNDY (SEQ ID NO:10)	(13aa)	1569	7.1	Scrambled version of A1
A1/2	EEEEGGGGYYVNWYFDEEE (SEQ ID NO:11)	(18aa)	2244	5.8	Acidic pI, soluble,
A1/3	RRREGGGGGYYVNWYFDRRR (SEQ ID NO:12)	(18aa)	2407	9.9	Basic pI, soluble,
A1/4	EYGEYGEVNEYDEFWE (SEQ ID NO:13)	(18aa)	2244	5.8	Scrambled version of A1/2
A1/5	VRYNRYRWGYRGRFGDE (SEQ ID NO:14)	(18aa)	2407	9.9	Scrambled version of A1/3
A1/3-scr3	RRRGEYGVYWNGDFYRRR (SEQ ID NO:15)	(18aa)	2407	9.9	Scrambled version of A1/3
A1/3-Rd	RdRdRdEGGGYYVNWYFDRdRdRd	(18aa)	2407	9.9	Peptide A1/3 but substitute D-Arg for L-Arg
A1/3-Rd-srmb	RdRdRdGEYGVYWNGDFYRdRdRd	(18aa)	2407	9.9	Scrambled version of A1/3-Rd

Table 2

List of a series of antibody 198/A1 derived peptides. Listed are the length of the peptide (aa, amino acids #), the calculated molecular weight (MW, in Dalton (D)) and the statistical isoelectric point (pI). D-Arg is abbreviated as Rd.

In a first series of experiments we improved the solubility of the original CDR3H peptide sequence (A1; EGGGYYVNWYFDV; SEQ ID NO:8) by removing the C-terminal Val residue and adding several charged residues at the N— as well as the C-terminal end of the peptide. The resulting peptides, A1/2 (acidic pI), A1/3 (basic pI) and their respective scrambled versions A1/4, A1/5 and A1/3scr3 were readily soluble in a variety of buffer systems at physiological pH.

To analyze the FVIII-like (FIXa activating) activity of the peptides, an assay system based on a commercial available

peptide solution in the microtiter plate and incubated at 37° C. Absorbance at 405 nm and 490 nm of the samples was read at various times (5 min to 2 h) against a reagent blank in a Labsystems iEMS Reader MF™ microtiter plate reader using GENESIS™ software.

The result of this experiment are shown in Example 11, FIG. 18. Peptide A1/3 induced a readily measurable FXa generation in the presence of 2.9 nM human FIXa, whereas the scrambled version A1/5 was inactive. In addition, the acidic peptide A1/2 as well as the scrambled versions A1/4 and A1/3-scr3 did not give any significant chromogenic activity when tested under comparable conditions (data not shown). To prove that the peptide A1/3 like the parental antibody 198/A1 does not react with bovine FIXa and FX the experiment shown in FIG. 19 was done. The peptide

## US 7,033,590 B1

23

A1/3 was incubated as described above with (A1/3 (24  $\mu$ M), +hFIXa) and without (A1/3 (24  $\mu$ M), w/o hFIXa) 2.3 nM human FIXa (hFIXa). In a control experiment we added plain dilution buffer (IZ) supplemented with 2.3 nM hFIXa to the reaction mixture. As shown in FIG. 19, the reaction takes place only in the presence of human FIXa.

FIG. 18 demonstrates the chromogenic FVIII-like activity of peptide A1/3 in the presence of 2.9 nM human FIXa (hFIXa). The scrambled version of peptide A1/3, peptide A1/5 does not give rise to any FXa generation. FIG. 19 demonstrates the dependence of the chromogenic FVIII-like activity of peptide A1/3 on the presence of human FIXa (hFIXa). In the absence of human FIXa, peptide A1/3 does not give rise to any FXa generation. The buffer control, plain imidazole buffer is designated IZ.

The peptides were also analyzed for their potential to reduce the clotting time in a FVIII deficient plasma. The aPTT based one stage clotting assay was essentially done as described (see example 6). Clotting times (time from starting the reaction to the "clot"-formation) were compared either against FVIII, a buffer control (IZ) or a control peptide (scrambled version). The results of two typical clotting experiments done with two different aPTT reagents (DAPTTIN and Pathromtin SL) are shown in table 3A and table 3B.

	peptide conc.	w/o FIXa sec	w/o FIXa sec	average sec	2.2 nM FIXa sec	2.2 nM FIXa sec	average sec
Exp. 1							
IZ	0	107.7	106.8	107	93.1	94.5	94
A1/3	15 $\mu$ M	78.2	77.1	78	59.3	59.9	60
	12.5 $\mu$ M	80.2	80.6	80	60.2	58.9	60
	7.5 $\mu$ M	97.8	97.9	98	73.1	72.7	73
	2.5 $\mu$ M	105.2	104.8	105	91.1	91	91
A1/3-scr3	15 $\mu$ M	122.5	122	122	106.1	105.5	106
	12.5 $\mu$ M	116	117.6	117	103.1	104.5	104
	7.5 $\mu$ M	114.2	113.9	114	100.8	100.6	101
	2.5 $\mu$ M	107.8	107.4	108	96.3	95.2	96
Exp. 2							
IZ	0	111	109.7	110	94.7	95.5	95
A1/3	12.5 $\mu$ M	83.6	85.5	85	56.7	56.7	57
	10 $\mu$ M	79.1	78.5	79	63.1	62.5	63
	7.5 $\mu$ M	100.1	100.5	100	71.6	73.9	73
	5 $\mu$ M	103.4	104.8	104	77	76	77
	2.5 $\mu$ M	110.1	108.9	110	88	88.8	88
	1.25 $\mu$ M	108.7	109.3	109	90.7	90.8	91

Table 3A. Clotting activity of peptides A1/3 and A1/3-scr (scrambled version of A1/3) in FVIII deficient plasma either in the presence or in the absence (w/o) of 2.2 nM human FIXa. Shown are two independent representative experiments (Exp. 1 and Exp. 2). All clotting experiments have been done in duplicate. Given are the clotting times for the individual experiments and the average clotting time in seconds (sec). Experiments shown in table 3A have been done employing the aPTT reagent DAPTTIN (Baxter Hyland Immuno). Compared to the buffer control (IZ, imidazole buffer) the peptide A1/3 gave rise to a dose dependent reduction in the clotting time. The reduction in the clotting time became much more pronounced by the addition of 2.2 nM activated human FIX to the reaction mix. The scrambled version of peptide A1/3, A1/3-scr3 did not show any reduction of the clotting time. In fact, at concentrations above 2.5  $\mu$ M, the scrambled peptide became inhibitory and therefore prolonged the clotting time. Peptides A1/1, A1/2, A1/4 and A1/5 did not give any reduction in the

24

clotting time indicating that they lack procoagulant activity (data not shown).

	Final conc.	w/o FIXa sec	w/o FIXa sec	average sec	2.2 nM FIXa sec	2.2 nM FIXa sec	average sec
IZ	0	131.8	132.1	132	107.9	108.7	108
FVIII	12.5 mU/ml	68.9	69	69	52.9	53.6	53
	6.25 mU/ml	77.8	77.9	78	58.6	58.9	59
A1/3	15 $\mu$ M	152.8	149.3	151	75.4	75.2	75
	10 $\mu$ M	135.7	134.6	135	76.2	79.8	78
	5 $\mu$ M	152.6	155.6	154	86.6	90.2	88
	1 $\mu$ M	138.3	138.8	139	103.7	105.9	105

Table 3B. Clotting activity of peptide A1/3 in FVIII deficient plasma when Pathromtin SL (DADE Behring) is used as an aPTT reagent. The experiments were done in duplicate, either in the presence or in the absence (w/o) of 2.2 nM human FIXa. Given are the clotting times for the individual experiments and the average clotting time in seconds (sec). Factor VIII and imidazole buffer (IZ) were included as positive and negative control respectively.

In contrast to the experiments shown in table 3A the experiments shown in table 3B have been done employing the aPTT reagent Pathromtin SL. In the presence of FIXa, the peptide A1/3 gave rise to a dose dependent reduction in the clotting time whereas in the absence of FIXa no reduction of the clotting time was detectable.

In another series of experiments we set out to improve the plasma stability (protection from, e.g., proteolytic degradation) of peptide A1/3. One approach was to substitute the N- and C-terminal L-Arg residues with D-Arg residues (exemplified by peptides A1/3-rd and A1/3-Rd-srmb). Peptides A1/3-rd and A1/3-Rd-srmb (scrambled version of the peptide) were then analyzed in a chromogenic as well as in the aPTT based clotting assay. These experiments revealed that exchanging the terminal L-Arg residues for D-Arg residues did not change the FVIII-like activity as measured in the chromogenic assay, indicating that chirality of the Arg-residues does not play a major role in chromogenic activity (FIG. 20). In addition, the aPTT based one-stage clotting activity, although somewhat reduced, was still easily detectable (Table 4).

	Peptide conc.	w/o FIXa sec	w/o FIXa sec	average sec	2.2 nM FIXa sec	2.2 nM FIXa sec	average sec
IZ	0	110	109.1	110	96	96	96
A1/3	15 $\mu$ M	77.8	78	78	56.1	55.5	56
	12.5 $\mu$ M	99.4	100.5	100	65	68	67
	10 $\mu$ M	104.4	104.5	104	72	73.2	73
	7.5 $\mu$ M	105.2	105.2	105	80.7	80.5	81
	5 $\mu$ M	108.4	107.7	108	89.7	88.3	89
	2.5 $\mu$ M	107.9	107.6	108	93.6	93.3	93
A1/3-Rd	1.25 $\mu$ M	106.7	107	107	94.4	95	95
	15 $\mu$ M	96.4	95.4	96	76.1	74.4	75
	12.5 $\mu$ M	98	98.6	98	72.3	73.7	73
	10 $\mu$ M	93.5	95.8	95	74.2	77.2	76
	7.5 $\mu$ M	97.6	98.1	98	80.9	82.2	82
	5 $\mu$ M	99.2	99.1	99	86	85.1	86
A1/3-Rd-srmb	2.5 $\mu$ M	102.7	103.4	103	94.4	94.7	95
	1.25 $\mu$ M	107.5	107.7	108	96.6	96	96
	15 $\mu$ M	121.9	121.3	122	112.7	112.4	113
	12.5 $\mu$ M	117.2	118	118	108.1	107.8	108
	10 $\mu$ M	115.8	115.3	116	107.2	107.8	108
	7.5 $\mu$ M	114.6	113.6	114	107.6	106.6	107
	5 $\mu$ M	113.1	112.4	113	108.5	108.2	108

US 7,033,590 B1

25

-continued

Peptide conc.	w/o FIXa sec	w/o FIXa, sec	average sec	2.2 nM FIXa sec	2.2 nM FIXa sec	average sec
2.5 $\mu$ M	111.9	111.9	112	105	104.2	105
1.25 $\mu$ M	107.2	107.1	107	101.1	105.3	103

Table 4. One stage clotting activity of peptides A1/3, A1/3-Rd and A1/3-Rd-srmb (sequences see table 2). IZ, buffer control.

FIG. 20 demonstrates the unchanged chromogenic activity of peptide A1/3-Rd. Peptides at a final concentration of 12  $\mu$ M or the buffer control (IZ) were incubated in the presence of 2.3 nM human FIXa (+). The chromogenic activity of peptide A1/3 and A1/3-Rd was found to be virtually unchanged and gave almost identical results in the chromogenic assay. The scrambled version of peptide A1/3, A1/5 as well as the buffer gave no significant FXa generation.

In the next series of experiments we set out to determine the individual role of any amino acid of the peptide core sequence by substituting each residue for the amino acid Alanine (Table 5).

26

100 mM NaCl, 1% human albumin, pH7.4) to the desired final concentration. The peptides were analyzed for their chromogenic activity as well as for their potential to reduce the clotting time in a FVIII deficient plasma. The one-stage clotting assay was essentially done as described (see example 6). Clotting times (time from starting the reaction to the "clot"-formation) were compared either against a buffer control or a control peptide (scrambled version).

Some of the results of the "Alanine scan" are given for the peptides A1/3-2 and A1/3-3. The change of G<sub>3</sub>-A<sub>3</sub> as exemplified in the peptide A1/3-2 yields high chromogenic activity and a strong reduction of the one-stage clotting time (34 seconds at a concentration of 12.5  $\mu$ M) in the presence of 2.2 nM human FIXa. Peptide A1/3-3 (G<sub>4</sub>-A<sub>4</sub>) exhibits an optimum of chromogenic activity around a final concentration of 12  $\mu$ M with decreased activity at either higher or lower concentrations. The peptide is somewhat inhibitory in a one-stage clotting assay at higher concentrations (12.5  $\mu$ M) in the absence of FIXa but becomes strongly active in the presence of 2.2 nM FIXa (31 seconds, 12.5  $\mu$ M).

In the next series of experiments we set out to determine the individual role of any amino acid of the peptide core sequence by substituting each core residue for the amino acid glutamic acid (E) (see Table 6).

Peptide	Sequence	Amino acid #	MW (D)	pI	Remark
A1/3	RRREGGGYYVNWFDRRR (SEQ ID NO:12)	(18aa)	2407	9.9	Basic pI, soluble.
A1/3-13	RRRAGGGYYVNWFDRRR (SEQ ID NO:19)	(18aa)	2349	10.4	E <sub>1</sub> -A <sub>1</sub>
A1/3-1	RRREAGGGYYVNWFDRRR (SEQ ID NO:20)	(18aa)	2421	9.9	G <sub>2</sub> -A <sub>2</sub>
A1/3-2	RRREGAGYYVNWFDRRR (SEQ ID NO:21)	(18aa)	2421	9.9	G <sub>3</sub> -A <sub>3</sub>
A1/3-3	RRREGGAYVNWFDRRR (SEQ ID NO:22)	(18aa)	2421	9.9	G <sub>4</sub> -A <sub>4</sub>
A1/3-4	RRREGGGAYVNWFDRRR (SEQ ID NO:23)	(18aa)	2315	9.9	Y <sub>5</sub> -A <sub>5</sub>
A1/3-5	RRREGGGYAVNWFDRRR (SEQ ID NO:24)	(18aa)	2315	9.9	Y <sub>6</sub> -A <sub>6</sub>
A1/3-6	RRREGGGYYANWFDRRR (SEQ ID NO:25)	(18aa)	2379	9.9	V <sub>7</sub> -A <sub>7</sub>
A1/3-7	RRREGGGYYVAWFDRRR (SEQ ID NO:26)	(18aa)	2364	9.9	N <sub>8</sub> -A <sub>8</sub>
A1/3-8	RRREGGGYYVNAYFDRRR (SEQ ID NO:27)	(18aa)	2292	9.9	W <sub>8</sub> -A <sub>9</sub>
A1/3-9	RRREGGGYYVNWAFDRRR (SEQ ID NO:28)	(18aa)	2315	9.9	Y <sub>10</sub> -A <sub>10</sub>
A1/3-10	RRREGGGYYVNWYADRRR (SEQ ID NO:29)	(18aa)	2331	9.9	F <sub>11</sub> -A <sub>11</sub>
A1/3-11	RRREGGGYYVNWFARRR (SEQ ID NO:30)	(18aa)	2363	10.5	D <sub>12</sub> -A <sub>12</sub>
A1/3-12srmb	RRRYVYNGWGYFEGARRR (SEQ ID NO:31)	(18aa)	2363	10.4	Scrambled version

55

Table 5. Listed are the peptides designed to elucidate the role of any single amino acid within the peptide core sequence (E<sub>1</sub>G<sub>2</sub>G<sub>3</sub>G<sub>4</sub>Y<sub>5</sub>Y<sub>6</sub>V<sub>7</sub>N<sub>8</sub>W<sub>9</sub>Y<sub>10</sub>F<sub>11</sub>D<sub>12</sub>; SEQ ID NO:112). The subscripted numbers describe the position of the amino acid within the peptide. Alanine, an uncharged small amino acid, was substituted for each amino acid ("Alanine scan"). Also listed are the lengths of the peptides (amino acids #), the calculated molecular weights (MW, in Dalton (D)) and the statistical isoelectric points (pI).

Each of the peptides was dissolved individually in imidazole buffer (50 mM imidazole, 100 mM NaCl, pH7.2) and subsequently diluted in clotting buffer (50 mM imidazole,

Peptide	Sequence	Amino- Acids	MW (D)	pI	Remark
A1/3	RRREGGGYYVNWFDRRR (SEQ ID NO:12)	(18aa)	2407	9.9	Basic pI, soluble,
A1/3-22	RRREGGGYYVNWFDRRR (SEQ ID NO:32)	(18aa)	2479	9.5	G <sub>2</sub> -E <sub>2</sub>
A1/3-23	RRREGGGYYVNWFDRRR	(18aa)	2479	9.5	G <sub>3</sub> -E <sub>3</sub>

65



US 7,033,590 B1

27

28

-continued

Peptide	Sequence	Amino-Acids	MW (D)	pI	Remark
A1/3-24	(SEQ ID NO:33) RRREGGEYVNWYFDRRR	(18aa)	2479	9.5	G <sub>4</sub> -E <sub>4</sub>
A1/3-26	(SEQ ID NO:34) RRREGGEYVNWYFDRRR	(18aa)	2373	9.4	Y <sub>5</sub> -E <sub>5</sub>
A1/3-27	(SEQ ID NO:35) RRREGGEYVNWYFDRRR	(18aa)	2373	9.4	Y <sub>6</sub> -E <sub>6</sub>
A1/3-28	(SEQ ID NO:36) RRREGGEYVNWYFDRRR	(18aa)	2437	9.5	V <sub>7</sub> -E <sub>7</sub>
A1/3-29	(SEQ ID NO:37) RRREGGEYVNWYFDRRR	(18aa)	2422	9.5	N <sub>8</sub> -E <sub>8</sub>
A1/3-30	(SEQ ID NO:38) RRREGGEYVNWYFDRRR	(18aa)	2350	9.5	W <sub>9</sub> -E <sub>9</sub>
A1/3-31	(SEQ ID NO:39) RRREGGEYVNWYFDRRR	(18aa)	2373	9.4	Y <sub>10</sub> -E <sub>10</sub>
A1/3-32	(SEQ ID NO:40) RRREGGEYVNWYFDRRR	(18aa)	2389	9.5	F <sub>11</sub> -E <sub>11</sub>
A1/3-33	(SEQ ID NO:41) RRREGGEYVNWYFDRRR	(18aa)	2421	9.9	D <sub>12</sub> -E <sub>12</sub>
A1/3-34smb	(SEQ ID NO:42) RRREGGEYVNWYFDRRR	(18aa)	2437	9.5	Scrambled version

Table 6. Listed are the peptides designed to elucidate the role of any single amino acid within the peptide core sequence (E<sub>1</sub>G<sub>2</sub>G<sub>3</sub>G<sub>4</sub>Y<sub>5</sub>Y<sub>6</sub>V<sub>7</sub>N<sub>8</sub>W<sub>9</sub>Y<sub>10</sub>F<sub>11</sub>D<sub>12</sub>; SEQ ID NO:112). The subscripted numbers describe the position of the amino acid within the peptide. Glutamic acid, a negatively charged large amino acid, was substituted for each amino acid of the core sequence ("Glutamic acid scan"). Also listed are the lengths of the peptide (amino acids #), the calculated molecular weights (MW, in Dalton (D)) and the statistical isoelectric points (pI).

Each of the peptides was solved individually in imidazole buffer (50 mM imidazole, 100 mM NaCl, pH7.2) and subsequently diluted in clotting buffer (50 mM imidazole, 100 mM NaCl, 1% human albumin, pH7.4) to the desired final concentration. The peptides derived from the "Glutamic acid scan" series were analyzed for their chromogenic FVIII-like activity as well as for their potential to reduce the clotting time in a FVIII deficient plasma. The one-stage clotting assay was essentially done as described (see example 6).

The peptide A1/3-24 showed some interesting properties. The molecule exhibited high chromogenic FVIII-like activity at concentrations between 6.5  $\mu$ M-12  $\mu$ M but lost activity at higher concentrations (up to 24  $\mu$ M). The peptide had no procoagulant activity in the absence of human FIXa but was strongly active in the presence of 2.2 nM hFIXa.

In a second series of experiments we set out to improve the procoagulant activity of the antibody-198/B1 CDR3H derived peptide sequence B1. In a first step we improved the solubility of the original peptide sequence (B1; EGGGFTVNWYFDV; SEQ ID NO:7) by removing the C-terminal Val residue and adding several charged residues at the N- as well as the —C-terminal end of the peptide. The resulting peptides B1/4, B1/6 (acidic pI), B1/7 (basic pI) and their scrambled versions B1/5, B1/7scr3 are readily soluble in a variety of buffer systems at physiological pH.

Peptide	Sequence	Amino-acids	MW (D)	pI	Remark
B1	EGGGFTVNWYFDV	(13aa)	1491	6.0	Decreased solubility
B1/4	(SEQ ID NO:7) REGGGFTVNWYFDR	(14aa)	1704	7.9	Soluble,
B1/5	(SEQ ID NO:45) FGVGYRGETRNFWD	(14aa)	1704	8.0	Scrambled version, soluble
B1/6	(SEQ ID NO:46) EEEEGGGFTVNWYFDEEE	(18aa)	2166	5.0	Acidic pI soluble
B1/7	(SEQ ID NO:47) RRREGGGFTVNWYFDRRR	(18aa)	2329	9.9	Basic pI soluble
B1/7scr3	(SEQ ID NO:48) RRRFGVGYGETNFDWRRR	(18aa)	2329	9.9	Basic pI, soluble, scrambled version

Table 7 is a list of a series of antibody 198/B1 derived peptides. Listed are the length of the peptide (aa, amino acids #), the calculated molecular weight (MW, in Dalton (D)) and the statistical isoelectric point (pI).

Peptides B1/4 and B1/5 were soluble in 50 mM Tris, 100 mM NaCl, pH=6.5. Both peptides were analyzed in a chromogenic FVIII assay. Peptide B1/4 but not the scrambled version B1/5 was found to have some chromogenic activity (data not shown).

Subsequently peptides B1/6, B1/7 and B1/7scr3 were analyzed. Each of the peptides was solved individually in 50 mM imidazole, 100 mM NaCl, pH7.2 and subsequently diluted either in clotting buffer (50 mM imidazole, 100 mM NaCl, 1% human albumin, pH7.4) or in imidazole buffer to the desired final concentration. The peptides were analyzed for their chromogenic activity as well as for their potential to reduce the clotting time in a FVIII deficient plasma (table 8 & 9). The one stage clotting assay was essentially done as described (see example 6). Clotting times (time from starting the reaction to the "clot"-formation were compared either against a buffer control or a control peptide (scrambled version).

The FIXa activating activity (FVIII cofactor-like activity) from peptide B1/7 was first measured in the chromogenic assay described above.

As shown in FIG. 21, the addition of 2.4  $\mu$ M peptide B1/7 to the reaction mixture led to a well measurable generation of FXa. In contrast, the addition of 35  $\mu$ M Pefabloc Xa, a specific inhibitor of FXa protease activity, resulted in a significant reduction of the chromogenic substrate cleavage reaction (FIG. 22) thereby proving that there was indeed a peptide-FIXa mediated FXa generation. If there was no addition of FIXa and FX to the reaction mixture, no FXa was synthesized (FIG. 22). Peptide B1/6 and the control peptides B1/5 and B1/7scr3 exhibited no activity (data not shown).

FIG. 21 demonstrates the chromogenic activity of peptide B1/7. The peptide at a final concentration of 2.4  $\mu$ M or the buffer control (IZ) were incubated in the presence of 2.3 nM human FIXa.

In FIG. 22 peptide B1/7 at a final concentration of 2.4  $\mu$ M or the buffer control (IZ) were incubated in the presence of 2.3 nM human FIXa (as indicated either as "+2.3 nM hFIXa" or "+") The chromogenic activity of peptide B1/7 was found to be dependent on the presence of FIXa and FX since no reaction is detectable when FIXa and FX are left out of the reaction (w/o FIXa/FX). To prove that the peptide B1/7 mediates indeed FXa generation, the FXa specific protease inhibitor Pefabloc Xa was added to the reaction mix (35  $\mu$ M Pefabloc Xa). In a second set of experiments, the procoagu-

## US 7,033,590 B1

29

lant effect of peptides B1/6, B1/7 and B1/7scr3 were tested in a aPTT based one-step coagulation assay. The experiments were done essentially as described in Example 6. The results are shown in tables 8 and 9.

Pep- tide	12.5 $\mu$ M (-)	1.25 $\mu$ M (-)	0.125 $\mu$ M (-)	12.5 nM (-)	Buffer (-)	remarks
B1/6	115	110	111	111	110	
B1/7	157	112	109	110	110	
B1/7 scr3	115	105	106	105	107	

Table 8: FVIII deficient plasma was incubated either with peptides B1/6, B1/7scr3 or B1/7 in the absence of activated human FIX. As a negative control, plain buffer was added to the deficient plasma. The clotting times for the various combinations are given. Under these conditions, peptide B1/7 at its highest concentration (12.5  $\mu$ M) becomes inhibitory to the coagulation process as indicated by the extended clotting time of 157 seconds.

Pep- tide	12.5 $\mu$ M (+)	1.25 $\mu$ M (+)	0.125 $\mu$ M (+)	12.5 nM (+)	Buffer (+)	remarks
B1/6	103	100	101	100	100	
B1/7	83	92	99	99	100	
B1/7 scr3	102	94	94	94	94	

Table 9: FVIII deficient plasma was incubated either with peptides B1/6, B1/7scr3 or B1/7 in the presence of activated human FIX. As a negative control, plain buffer was added to the deficient plasma. The clotting times for the various combinations are given. In the presence of FIXa, peptide B1/7 becomes procoagulant as indicated by the reduced clotting time (83 seconds compared to 102 seconds for the scrambled peptide and 100 seconds for the buffer control).

## Example 12

#### Procoagulant Activity of Peptide Derivatives Obtained from CDR3 Regions of Anti-FIX/FIXa- Antibodies in FVIII Inhibitor Plasma

To assay for the procoagulant activity of peptide A1/3 in FVIII inhibitor plasma the following experiment was carried out. We performed a standard aPTT based one stage clotting assay, but instead of FVIII deficient plasma we employed FVIII inhibitor plasma. The inhibitory potency of the plasma was 8.1 Bethesda Units per ml.

TABLE 10

	Peptide conc.	w/o FIXa sec	w/o FIXa sec	Average sec	FIXa sec	FIXa sec	average sec
IZ	0	104.9	103.6	104	94.2	94.1	94
A1/3	12.5 $\mu$ M	85.8	85.3	86	61	60.2	61
	10 $\mu$ M	88.4	87.9	88	61.3	61.8	62
	7.5 $\mu$ M	93.7	92.7	93	68.8	70.9	70
	5 $\mu$ M	101.5	101.1	101	81	82	82
	2.5 $\mu$ M	106.1	105.3	106	90.2	90.5	90
	1.25 $\mu$ M	104.5	104.3	104	91.3	91.4	91

Table 10: Various amounts of peptide A1/3 (12.5  $\mu$ M-1.25  $\mu$ M) were added to FVIII inhibitor plasma (either in the

30

presence (FIXa) of 2.2 nM FIXa or in the absence (w/o FIXa). As a negative control, plain buffer was added to the plasma (IZ). Experiments were done in duplicate and the average (aver.) was calculated. The clotting times (in seconds) for the various combinations are given. It is easily appreciable that the peptide A1/3 reduces (in a dose dependent manner) the clotting time of FVIII inhibitor plasma in the presence of FIXa but, although albeit to a much lesser extent, also in the absence of FIXa.

## Example 13

#### Conversion of the 196/C4 IgM into IgG1

Since some IgM antibodies demonstrate high FVIII-like activity in chromogenic assays, attempts were made to convert such IgM antibodies into IgG antibodies (though antibody derivatives such as Fab, F(ab)<sub>2</sub>, scFv, etc. could also be produced). Described in detail below is the rescue of the IgM variable region genes. Expression vector pBax-IgG1 (FIG. 23) was first constructed from vectors pSI (Promega) and pEF/Bsd (Invitrogen) through multiple cloning steps. B-lymphocytes of a donor are purified from blood and mature mRNA purified from these cells using the "micro-mRNA purification-kit" (Pharmacia). The cDNA of a human kappa chain and a human gamma 1 chain are prepared employing the "you-primefirst-strand-cDNA-"kit" (Pharmacia) using specific primers.

The coding sequence of a human kappa light chain constant domain is amplified from the cDNA by PCR using specific primers.

The gene of a human gamma 1 chain constant region (CH1-hinge-CH2-CH3) is amplified from the cDNA by PCR using specific primers.

The PCR product of the light chain constant domain is digested with XbaI and NheI and inserted into digested pSI. The resultant vector is cleaved with EcoRI and XbaI and annealed oligonucleotides are inserted, resulting in vector pSI-Ckappa. The annealed oligonucleotides provide for the leader and the SacI-XbaI sites for insertion of the kappa chain variable region. The PCR product of the human gamma 1 chain constant region is digested with SpeI and BamHI and inserted into digested pSI. The resultant vector is cleaved with SpeI and NotI and annealed oligonucleotides are inserted resulting in vector pSI-Cgamma. The annealed oligonucleotides provide for the leader and the XhoI-BstEI sites for insertion of the heavy chain variable region. Vector pEF/Bsd is digested NheI and SfiI, blunt ended by Klenow treatment and the whole expression cassette of pSI-Ckappa, excised with BglII and BamHI, is inserted (after Klenow treatment). The resultant vector is digested with EcoRI and HindIII and treated with Klenow. The whole expression cassette of pSI-Cgamma is excised with BglII and BamHI and is inserted (after Klenow treatment). The resultant vector is named pBax-IgG1.

The light chain variable region can be inserted in between the SacI-XbaI sites, yielding the complete coding-sequence of a kappa light chain. The heavy chain variable region can be cloned in between the XhoI-BstEI sites, resulting in a complete IgG1 heavy chain gene. Both open reading frames are expressed under the control of the SV40-promoter and harbour the coding sequence of a signal peptide at the 5' end of the genes for secretion of the heavy and light chains into the endoplasmatic reticulum. Transfection into COS cells allows the expression of an IgG1 with the same binding properties as the parental IgM.

Construction of the plasmid pBax-196/C4 is further accomplished by amplifying the VH of the 196/C4 scFv

US 7,033,590 B1

31

(subcloned as described in Experiment 10) by PCR using specific primers. The PCR product is digested with XhoI and BstEII and inserted into XhoI and BstEII digested pBax IgG1. The VL of the 196/C4 scFv is amplified by PCR using specific primers. The PCR product is digested with SacI and XbaI and inserted into SacI and XbaI-digested pBax IgG1-VH. The resultant vector (pBax-196/C4) is transfected into COS cells by electroporation, and hybrid IgG1 molecules (murine variable region and human constant region) with the same specificity as the parental IgM is expressed.

## Example 14

## Activation of FIXa Amydolytic Activity by Anti-FIXa Antibodies

Briefly, 20±1 factor IXa (containing 20 mU FIXa (Stago)) were incubated at 37° C., with 200 µl of reaction buffer (50 mM Tris HCl pH7.4, 100 mM NaCl, 5 mM CaCl<sub>2</sub> and 40% Ethyleneglycol), 25 µl of FIXa substrate (CH<sub>3</sub>SO<sub>2</sub>-D-CHG-Gly-Arg-pNA, AcOH, 10M/ml, Pentapharm LTD) in the absence or presence of various amounts of anti-FIX antibodies 198/B1 (IgG isotype) or 196/AF1 (IgM isotype). Specific cleavage of FIXa substrate was monitored at 405 nm in an ELISA reader.

The presence of the anti-FIX antibodies enhanced the amydolytic activity of FIXa at least 2 fold. FIG. 24 shows the increase of the amidolytic activity of FIXa in the presence of antibody 198/B1 (FIG. 24A) and antibody 198/AF1 (FIG. 24B).

## Example 15

## FVIII-like Activity Exhibited by Fab Fragments Derived from Anti FIX/FIXa-antibodies

Fab fragments of anti-FIX/FIXa antibodies were prepared and purified according to standard protocols. Briefly, 1 ml antibody 198/A1 (4 mg/ml in 50 mM imidazole, 100 mM NaCl, pH7.4) was incubated overnight with 87 µl fragmentation buffer (1M Na Acetate, 10 mM EDTA 67.5 mg/ml L-cysteine) and 0.25 mg papain (immobilized on agarose beads), at 37° C. The preparation was filtered to remove the papain. L-histidine was added (final concentration 50 mM) and afterwards the pH was adjusted to 7.0. Finally, solid NaCl is added to give a final concentration of 1M.

Subsequently, the 198/A1 Fab fragment was purified by binding to protein L: we used ImmunoPure Immobilized PROTEIN L Plus (Pierce) in a PHARMACIA XK 16/20 Column (gel-volume: 2 ml) Buffers for chromatography were: 1) equilibration-buffer: 50 mM L-histidine pH 7.0; 1M NaCl; 0.1% (w/v) NaN<sub>3</sub>; 2) wash-buffer: 50 mM L-Histidine pH 7.0; 0.1 (w/v) NaN<sub>3</sub>; 3) elution-buffer: 100 mM glycine pH 2.5; 0.1% (w/v) NaN<sub>3</sub>; and 4) neutralization buffer: 2M Tris/Cl pH 8.0;

Chromatography was essentially done by following steps 1 to 7 described in table 11. In order to neutralize the low pH of the elution buffer "Fraction-tubes" were pre-loaded with 0.2 ml 2M Tris pH 8.0.

TABLE 11

STEP	BUFFER	Flow rate	Vol.	CV	Fractions
1.	column-elution-wash	2.0 ml/min	10 ml	5	waste
2.	equilibration buffer	2.0 ml/min	10 ml	5	waste

32

TABLE 11-continued

STEP	BUFFER	Flow rate	Vol.	CV	Fractions
3.	sample-load	1.0 ml/min	x ml	x	flow-through
4.	wash 1	1.0 ml/min	20 ml	10	flow-through
5.	wash 2	1.0 ml/min	10 ml	5	flow-through
6.	elution	1.0 ml/min	15 ml	7.5	1.0 ml fractions-
7.	neutralization	2.0 ml/min	10 ml	5	waste

## Table 11

The final 198/A1 Fab preparation was dialyzed against 50 mM imidazole, 100 mM NaCl, pH7.4 and analyzed in a chromogenic FVIII assay as described above (FIG. 25). Compared to an intact antibody, the 198/A1 Fab fragment has somewhat less activity; however, the Fab fragment still gives rise to FIX dependent FXa generation.

FIG. 25 demonstrates the chromogenic FVIII-like activity of the antibody 198/A1 Fab fragment in the presence of 2.3 nM human FIXa. As a positive control we used the intact antibody 198/A1 as well as 7.5 pM FVIII. Buffer control (IZ) instead of 198/A1 Fab fragment or FVIII was used as a negative control.

## Example 16

FVIII-like Activity Exhibited by Fusion Proteins Between scFv Fragments of Anti-FIX/FIXa Antibodies and *E. coli* Alkaline Phosphatase

The single chain Fv fragment (see example 10) of antibody 198/B1 (subclone AB2) was fused to the N-terminus of *E. coli* alkaline phosphatase employing the pDAP2 vector system (Kerschbaumer et al., 1996). Two identical clones were isolated and designated pDAP2-198AB2#1 and pDAP2-198AB2#100 (FIG. 26). The resulting fusion proteins were expressed in *E. coli*, purified by metal affinity chromatography (Kerschbaumer et al., 1997) and analysed in a standard chromogenic assay (FIG. 27).

FIG. 27 demonstrates the chromogenic FVIII-like activity of two antibody 198/B1 (subclone AB2) scFv fragment-alkaline phosphatase fusion proteins (198AB2#1 and 198AB2#100) in the presence of 2.3 nM human FIXa. As a positive control we used 7.5 pM FVIII.

## Example 17

## FVIII-like Activity Exhibited by a Bivalent Miniantibody

In order to obtain a bivalent miniantibody, the scFv fragment of antibody 198/B1 (subclone AB2) was fused to a amphipatic helical structure employing the pZip1 vector system (Kerschbaumer et al. (Analytical Biochemistry 249, 219-227, 1997). Briefly, the gene of the 198/B1 scFv fragment was isolated from the plasmid pDAP-198AB2#100 (example 16) by digestion with SfiI and NotI. The DNA fragment was gel purified and inserted in the SfiI/NotI digested vector pZip1. The resulting plasmid was sequenced and designated pZip-198AB2#102 (FIG. 28). In parallel, we constructed a miniantibody version from an irrelevant monoclonal antibody termed #8860. In a first step, the single chain Fv fragment of antibody #8860 was assembled in the vector pDAP2. The cloning was done essentially as described in example 10. The construct was

US 7,033,590 B1

33

named pDAP2-8860scFv#11 (FIG. 29). Subcloning of the scFv fragment contained within pDAP2-8860scFv#11 into plasmid pZip1 (see above) yielded the miniantibody construct p8860-Zip#1.2 (FIG. 30). Since antibody #8860 does not react with FIX/FIXa (as judged by Western Blot and ELISA analysis) it represents an appropriate negative control. Subsequently, the miniantibody proteins were expressed in *E. coli* and purified from bacterial supernatants by binding to Protein L according to the following protocol: For affinity chromatography we used ImmunoPure Immobilized PROTEIN L Plus (Pierce) in a PHARMACIA XK 16/20 Columns having a gel-volume of 4 ml. Buffers employed were: 1) equilibration-buffer: 5 mM L-Histidine pH 7.0, 1M NaCl, 0.1% (w/v) NaN<sub>3</sub>, wash-buffer: 50 mM L-histidine pH 7.0, 0.1% (w/v) NaN<sub>3</sub>; elution-buffer: 100 mM glycine pH 2.5, 0.1% (w/v) NaN<sub>3</sub>; and neutralization buffer: 2M Tris/Cl pH 8.0.

Samples were prepared as follows: The bacterial culture supernatant was obtained by centrifugation of the bacterial expression culture (11,000×g, 4° C., 10 minutes). 470 g of ammonium-sulphate was added to 1 liter of supernatant and the solution stirred on ice for 1 hour to precipitate the protein. The precipitate was pelleted at 14,000×g for 35 minutes at 2° C. and re-dissolved in 100 ml 20 mM Tris pH 7.0. Subsequently the concentrate was dialyzed against 20 mM Tris pH 7.0, L-histidine was added to a final concentration of 50 mM and the pH was adjusted to 7.0. Finally, solid NaCl was added to give a final concentrations of 1M. Before loading on the column, a sample was first centrifuged at 16,000×g for 15 min at room temperature and then filtered through a 0.45 µm sterile filter.

Chromatography was essentially done by following steps 1 to 7 described in table 12. In order to neutralize the low pH of the elution buffer "Fraction-tubes" were pre-loaded with 0.2 ml 2M Tris pH 8.0.

STEP	BUFFER	Flow rate	Vol.	CV	Fractions
1. column-wash	elution-buffer	2.0 ml/min	20 ml	5	waste
2. equilibration	equi-buffer	2.0 ml/min	20 ml	5	waste
3. sample-load	sample	1.0 ml/min	x ml	x	flow-through
4. wash 1	equi-buffer	1.0 ml/min	40 ml	10	flow-through
5. wash 2	wash-buffer	1.0 ml/min	20 ml	5	flow-through
6. elution	elution-buffer	1.0 ml/min	30 ml	7.5	1.0 ml fractions-
7. neutralization	wash-buffer	2.0 ml/min	20 ml	5	waste

Table 12. The final 198/B1 (subclone AB2) miniantibody preparation (designated 198AB-Zip#102) and the negative control 8860-Zip#1.2 were dialyzed against 50 mM imidazole, 100 mM NaCl, pH7.4 and analyzed in a chromogenic FVIII assay as described above (FIG. 31).

As can be seen in FIG. 31, the miniantibody construct 198AB-Zip#102 gives rise to substantial FXa generation (compare to FVIII) whereas the negative control miniantibody 8860-Zip#1.2 does not.

FIG. 31 demonstrates the chromogenic FVIII-like activity of the 198/B1 (subclone AB2) miniantibody 198AB-Zip#102 in the presence of 2.3 nM human FIXa. As a positive control we used 4.8 pM FVIII whereas an unrelated miniantibody (8860-Zip#1.2) and plain reaction buffer (IZ) served as negative controls.

#### Example 18

##### FVIII-like Activity Exhibited by Anti-FIXa/FIX Antibody scFv Fragments

The single chain Fv fragment of antibody 198/B1 (subclone AB2) as well as the scfv fragment of antibody

34

#8860 were expressed employing the pMycHis6 vector system. Vector pMycHis6 (FIGS. 32 & 33) was constructed by cleaving vector pCOCK (Engelhardt et al., 1994, Biotechniques, 17: 44–46) with NotI and EcoRI and insertion of the following oligonucleotides: mychis6-co: 5' ggc-cgcagacacacaaactcatctcagaagaggatct gaatggggcgccacatcacatcaccatcactaataag 3' (SEQ. ID.NO.

79) and mychis-ic: 5'aattcttattagtgtggtgatggtgat-gtgcgcgcceccattcagatcctct tctgagatgagttttgttctgc 3' (SEQ.ID.NO. 80) FIG. 32 shows a schematic representation of the plasmid pMycHis6. The c-myc-tag sequence is used to detect the scFv fragment in an ELISA or a Western Blot analysis (Evan et al., Mol.Cell.Biol., 1985, 5(12), pp. 3610–6). The His6-tag sequence was included to facilitate the purification of scFv fragments by metal ion chromatography (Hochuli et al., 1988, Biotechnology, 6: 1321–1325). The plasmid contains the lacZ gene promoter (PlacZ) the PelB-leader sequence (see legend FIG. 26) an *E. coli* origin of replication (colElori) and a M13 phage origin of replication (M13ori). To allow for specific selection, the plasmid also carries the gene for the enzyme β-lactamase (AmpR) mediating resistance against the antibiotic ampicillin.

The gene of the 198/B1 (clone AB2)-scFv was rescued from plasmid pDAP2-198AB2#100 (example 16) by digestion with SfiI and NotI and inserted into SfiI/NotI cleaved pMycHis6. The resultant plasmid was designated pMycHis-198AB2#102. FIG. 34 shows the nucleotide and amino acid sequence of 198AB2 scFv (linked to the c-myc-tag and the His6tag):the resulting ORF of the expression vector is named pMycHisG-198AB2#102. Vector pMycHis6 was constructed by cleaving vector pCOCK (Engelhardt O. et al, BioTechniques 17, 44–46, 1994) NotI-EcoRI and inserting the following annealed oligonucleotides: (5'-G G C C G C A G A A C A A A A A C T C A T C T C A G A A G A G G A T C T G A A T G G G G C G G C A C A T C A C C A T C A C C A T C A C T A A T A A G -3' (SEQ.ID.No. 103) and 5'-T T A T T A G T G A T G G T G A T G G T G A T G T G C C G C C C C A T T C A G A T C T C T T C T G A G A T G A G T T T T G T T C T G C -3'(SEQ.ID.NO. 104)). The resultant vector, named pMycHis6, was cleaved SfiI-NotI and the gene of scFv 198AB2 was swapped into this vector from vector pDAP2-198AB2#100.

In analogy to the 198AB2 construct, the #8860 scFv fragment was cloned from a plasmid designated pDAP2-8860scFv clone 11. The pure scFv protein of #8860 was designated 8860-M/H#4c (plasmid p8860-M/H#4c, FIG. 35). The scFv proteins were expressed in *E. coli* and affinity purified from bacterial supernatants on Protein L columns (see example 17). The final MycHis-198AB2#102 and 8860-M/H#4c preparations were dialyzed against 50 mM imidazole, 100 mM NaCl, pH7.4 and analyzed in a chromogenic FVIII assay as described above (FIG. 36).

As can be seen in FIG. 36, the scfv construct MycHis-198AB2#102 gave rise to a substantial FXa generation whereas the negative controls 8860-M/H#4c and plain reaction buffer (IZ) did not.

FIG. 36 demonstrates the chromogenic FVIII-like activity of the 198/B1 (subclone AB2) scFv fragment (MycHis-198AB2#102) in the presence of 2.3 nM human FIXa. As a positive control we used 4.8 pM FVIII whereas an unrelated scfv (8860-M/H#4c) and plain reaction buffer (IZ) served as negative controls.

US 7,033,590 B1

35

36

## SEQUENCE LISTING

&lt;160&gt; NUMBER OF SEQ ID NOS: 112

&lt;210&gt; SEQ ID NO 1

&lt;211&gt; LENGTH: 26

&lt;212&gt; TYPE: DNA

&lt;213&gt; ORGANISM: Artificial Sequence

&lt;220&gt; FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence:primer  
oligonucleotide MOCG1-2FOR

&lt;400&gt; SEQUENCE: 1

ctcaatttttc ttgtccacct tgggtgc 26

&lt;210&gt; SEQ ID NO 2

&lt;211&gt; LENGTH: 26

&lt;212&gt; TYPE: DNA

&lt;213&gt; ORGANISM: Artificial Sequence

&lt;220&gt; FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence:primer  
oligonucleotide MOCG3FOR

&lt;400&gt; SEQUENCE: 2

ctcgatttctc ttgatcaact cagtct 26

&lt;210&gt; SEQ ID NO 3

&lt;211&gt; LENGTH: 24

&lt;212&gt; TYPE: DNA

&lt;213&gt; ORGANISM: Artificial Sequence

&lt;220&gt; FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence:primer  
oligonucleotide MOCMFOR

&lt;400&gt; SEQUENCE: 3

tggaatgggc acatgcagat ctct 24

&lt;210&gt; SEQ ID NO 4

&lt;211&gt; LENGTH: 24

&lt;212&gt; TYPE: DNA

&lt;213&gt; ORGANISM: Artificial Sequence

&lt;220&gt; FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence:primer  
MOCKFOR

&lt;400&gt; SEQUENCE: 4

ctcattcctg ttgaagctct tgac 24

&lt;210&gt; SEQ ID NO 5

&lt;211&gt; LENGTH: 10

&lt;212&gt; TYPE: PRT

&lt;213&gt; ORGANISM: Artificial Sequence

&lt;220&gt; FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence:hybridoma  
cell line 193/AD3 heavy chain CDR3 region

&lt;400&gt; SEQUENCE: 5

Tyr Gly Asn Ser Pro Lys Gly Phe Ala Tyr  
1 5 10

&lt;210&gt; SEQ ID NO 6

&lt;211&gt; LENGTH: 12

&lt;212&gt; TYPE: PRT

&lt;213&gt; ORGANISM: Artificial Sequence

&lt;220&gt; FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence:hybridoma  
cell line 193/K2 heavy chain CDR3 region

US 7,033,590 B1

37

38

-continued

---

<400> SEQUENCE: 6

Asp Gly Gly His Gly Tyr Gly Ser Ser Phe Asp Tyr  
 1 5 10

<210> SEQ ID NO 7

<211> LENGTH: 13

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence:hybridoma  
cell line 193/AB2 (derived from antibody 198/B1) heavy chain CDR3  
region, peptide B1

<400> SEQUENCE: 7

Glu Gly Gly Gly Phe Thr Val Asn Trp Tyr Phe Asp Val  
 1 5 10

<210> SEQ ID NO 8

<211> LENGTH: 13

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence:hybridoma  
cell line 198/A1 heavy chain CDR3 region, peptide A1

<400> SEQUENCE: 8

Glu Gly Gly Gly Tyr Tyr Val Asn Trp Tyr Phe Asp Val  
 1 5 10

<210> SEQ ID NO 9

<400> SEQUENCE: 9

000

<210> SEQ ID NO 10

<211> LENGTH: 13

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence:antibody  
198/A1 derived mutated peptide A1/1 scrambled version of A1

<400> SEQUENCE: 10

Val Tyr Gly Phe Gly Trp Gly Tyr Glu Val Asn Asp Tyr  
 1 5 10

<210> SEQ ID NO 11

<211> LENGTH: 18

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence:antibody  
198/A1 derived mutated peptide A1/2

<400> SEQUENCE: 11

Glu Glu Glu Glu Gly Gly Gly Tyr Tyr Val Asn Trp Tyr Phe Asp Glu  
 1 5 10 15

Glu Glu

<210> SEQ ID NO 12

<211> LENGTH: 18

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence:antibody  
198/A1 derived mutated peptide A1/3

US 7,033,590 B1

39

40

-continued

&lt;400&gt; SEQUENCE: 12

Arg Arg Arg Glu Gly Gly Tyr Tyr Val Asn Trp Tyr Phe Asp Arg  
 1 5 10 15

Arg Arg

&lt;210&gt; SEQ ID NO 13

&lt;211&gt; LENGTH: 18

&lt;212&gt; TYPE: PRT

&lt;213&gt; ORGANISM: Artificial Sequence

&lt;220&gt; FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence:antibody  
 198/A1 derived mutated peptide A1/4 scrambled version of A1/2

&lt;400&gt; SEQUENCE: 13

Glu Tyr Gly Glu Gly Tyr Gly Glu Val Asn Glu Tyr Asp Glu Phe Glu  
 1 5 10 15

Trp Glu

&lt;210&gt; SEQ ID NO 14

&lt;211&gt; LENGTH: 18

&lt;212&gt; TYPE: PRT

&lt;213&gt; ORGANISM: Artificial Sequence

&lt;220&gt; FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence:antibody  
 198/A1 derived mutated peptide A1/5 scrambled version of A1/3

&lt;400&gt; SEQUENCE: 14

Val Arg Tyr Arg Asn Arg Tyr Arg Trp Gly Tyr Arg Gly Arg Phe Gly  
 1 5 10 15

Asp Glu

&lt;210&gt; SEQ ID NO 15

&lt;211&gt; LENGTH: 18

&lt;212&gt; TYPE: PRT

&lt;213&gt; ORGANISM: Artificial Sequence

&lt;220&gt; FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence:antibody  
 198/A1 derived mutated peptide A1/3-scr3 scrambled version of A1/3

&lt;400&gt; SEQUENCE: 15

Arg Arg Arg Gly Glu Tyr Gly Val Tyr Trp Asn Gly Asp Phe Tyr Arg  
 1 5 10 15

Arg Arg

&lt;210&gt; SEQ ID NO 16

&lt;400&gt; SEQUENCE: 16

000

&lt;210&gt; SEQ ID NO 17

&lt;400&gt; SEQUENCE: 17

000

&lt;210&gt; SEQ ID NO 18

&lt;400&gt; SEQUENCE: 18

000

&lt;210&gt; SEQ ID NO 19

US 7,033,590 B1

41

42

-continued

---

```

<211> LENGTH: 18
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:antibody
      198/A1 derived mutant peptide A1/3-13 Alanine scan E-1-A-1

```

```

<400> SEQUENCE: 19

```

```

Arg Arg Arg Ala Gly Gly Gly Tyr Tyr Val Asn Trp Tyr Phe Asp Arg
 1             5             10             15

```

```

Arg Arg

```

```

<210> SEQ ID NO 20
<211> LENGTH: 18
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:antibody
      198/A1 derived mutant peptide A1/3-1 Alanine scan G-2-A-2

```

```

<400> SEQUENCE: 20

```

```

Arg Arg Arg Glu Ala Gly Gly Tyr Tyr Val Asn Trp Tyr Phe Asp Arg
 1             5             10             15

```

```

Arg Arg

```

```

<210> SEQ ID NO 21
<211> LENGTH: 18
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:antibody
      198/A1 derived mutant peptide A1/3-2 Alanine scan G-3-A-3

```

```

<400> SEQUENCE: 21

```

```

Arg Arg Arg Glu Gly Ala Gly Tyr Tyr Val Asn Trp Tyr Phe Asp Arg
 1             5             10             15

```

```

Arg Arg

```

```

<210> SEQ ID NO 22
<211> LENGTH: 18
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:antibody
      198/A1 derived mutant peptide A1/3-3 Alanine scan G-4-A-4

```

```

<400> SEQUENCE: 22

```

```

Arg Arg Arg Glu Gly Gly Ala Tyr Tyr Val Asn Trp Tyr Phe Asp Arg
 1             5             10             15

```

```

Arg Arg

```

```

<210> SEQ ID NO 23
<211> LENGTH: 18
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:antibody
      198/A1 derived mutant peptide A1/3-4 Alanine scan Y-5-A-5

```

```

<400> SEQUENCE: 23

```

```

Arg Arg Arg Glu Gly Gly Gly Ala Tyr Val Asn Trp Tyr Phe Asp Arg
 1             5             10             15

```

```

Arg Arg

```



US 7,033,590 B1

43

44

-continued

---

```

<210> SEQ ID NO 24
<211> LENGTH: 18
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:antibody
      198/A1 derived mutant peptide A1/3-5 Alanine scan Y-6-A-6

```

```

<400> SEQUENCE: 24

```

```

Arg Arg Arg Glu Gly Gly Gly Tyr Ala Val Asn Trp Tyr Phe Asp Arg
 1           5             10             15

```

```

Arg Arg

```

```

<210> SEQ ID NO 25
<211> LENGTH: 18
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:antibody
      198/A1 derived mutant peptide A1/3-6 Alanine scan V-7-A-7

```

```

<400> SEQUENCE: 25

```

```

Arg Arg Arg Glu Gly Gly Gly Tyr Tyr Ala Asn Trp Tyr Phe Asp Arg
 1           5             10             15

```

```

Arg Arg

```

```

<210> SEQ ID NO 26
<211> LENGTH: 18
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:antibody
      198/A1 derived mutant peptide A1/3-7 Alanine scan N-8-A-8

```

```

<400> SEQUENCE: 26

```

```

Arg Arg Arg Glu Gly Gly Gly Tyr Tyr Val Ala Trp Tyr Phe Asp Arg
 1           5             10             15

```

```

Arg Arg

```

```

<210> SEQ ID NO 27
<211> LENGTH: 18
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:antibody
      198/A1 derived mutant peptide A1/3-8 Alanine scan W-9-A-9

```

```

<400> SEQUENCE: 27

```

```

Arg Arg Arg Glu Gly Gly Gly Tyr Tyr Val Asn Ala Tyr Phe Asp Arg
 1           5             10             15

```

```

Arg Arg

```

```

<210> SEQ ID NO 28
<211> LENGTH: 18
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:antibody
      198/A1 derived mutant peptide A1/3-9 Alanine scan Y-10-A-10

```

```

<400> SEQUENCE: 28

```

```

Arg Arg Arg Glu Gly Gly Gly Tyr Tyr Val Asn Trp Ala Phe Asp Arg
 1           5             10             15

```

```

Arg Arg

```

US 7,033,590 B1

45

46

-continued

---

```

<210> SEQ ID NO 29
<211> LENGTH: 18
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:antibody
      198/A1 derived mutant peptide A1/3-10 Alanine scan F-11-A-11

```

```

<400> SEQUENCE: 29

```

```

Arg Arg Arg Glu Gly Gly Gly Tyr Tyr Val Asn Trp Tyr Ala Asp Arg
 1             5             10             15

```

```

Arg Arg

```

```

<210> SEQ ID NO 30
<211> LENGTH: 18
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:antibody
      198/A1 derived mutant peptide A1/3-11 Alanine scan D-12-A-12

```

```

<400> SEQUENCE: 30

```

```

Arg Arg Arg Glu Gly Gly Gly Tyr Tyr Val Asn Trp Tyr Phe Ala Arg
 1             5             10             15

```

```

Arg Arg

```

```

<210> SEQ ID NO 31
<211> LENGTH: 18
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:antibody
      198/A1 derived mutant peptide A1/3-12srmb scrambled version

```

```

<400> SEQUENCE: 31

```

```

Arg Arg Arg Tyr Val Tyr Asn Gly Trp Gly Tyr Phe Glu Gly Ala Arg
 1             5             10             15

```

```

Arg Arg

```

```

<210> SEQ ID NO 32
<211> LENGTH: 18
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:antibody
      198/A1 derived mutant peptide A1/3-22 Glutamic acid scan G-2-E-2

```

```

<400> SEQUENCE: 32

```

```

Arg Arg Arg Glu Gly Gly Gly Tyr Tyr Val Asn Trp Tyr Phe Asp Arg
 1             5             10             15

```

```

Arg Arg

```

```

<210> SEQ ID NO 33
<211> LENGTH: 18
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:antibody
      198/A1 derived mutant peptide A1/3-23 Glutamic acid scan G-3-E-3

```

```

<400> SEQUENCE: 33

```

```

Arg Arg Arg Glu Gly Gly Gly Tyr Tyr Val Asn Trp Tyr Phe Asp Arg
 1             5             10             15

```

```

Arg Arg

```

US 7,033,590 B1

47

48

-continued

---

```

<210> SEQ ID NO 34
<211> LENGTH: 18
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:antibody
      198/A1 derived mutant peptide A1/3-24 Glutamic acid scan G-4-E-4

```

```

<400> SEQUENCE: 34

```

```

Arg Arg Arg Glu Gly Gly Glu Tyr Tyr Val Asn Trp Tyr Phe Asp Arg
 1           5           10           15

```

```

Arg Arg

```

```

<210> SEQ ID NO 35
<211> LENGTH: 18
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:antibody
      198/A1 derived mutant peptide A1/3-26 Glutamic acid scan Y-5-E-5

```

```

<400> SEQUENCE: 35

```

```

Arg Arg Arg Glu Gly Gly Gly Glu Tyr Val Asn Trp Tyr Phe Asp Arg
 1           5           10           15

```

```

Arg Arg

```

```

<210> SEQ ID NO 36
<211> LENGTH: 18
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:antibody
      198/A1 derived mutant peptide A1/3-27 Glutamic acid scan Y-6-E-6

```

```

<400> SEQUENCE: 36

```

```

Arg Arg Arg Glu Gly Gly Gly Tyr Glu Val Asn Trp Tyr Phe Asp Arg
 1           5           10           15

```

```

Arg Arg

```

```

<210> SEQ ID NO 37
<211> LENGTH: 18
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:antibody
      198/A1 derived mutant peptide A1/3-28 Glutamic acid scan V-7-E-7

```

```

<400> SEQUENCE: 37

```

```

Arg Arg Arg Glu Gly Gly Gly Tyr Tyr Glu Asn Trp Tyr Phe Asp Arg
 1           5           10           15

```

```

Arg Arg

```

```

<210> SEQ ID NO 38
<211> LENGTH: 18
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:antibody
      198/A1 derived mutant peptide A1/3-29 Glutamic acid scan N-8-E-8

```

```

<400> SEQUENCE: 38

```

```

Arg Arg Arg Glu Gly Gly Gly Tyr Tyr Val Glu Trp Tyr Phe Asp Arg
 1           5           10           15

```

US 7,033,590 B1

49

50

-continued

Arg Arg

<210> SEQ ID NO 39  
 <211> LENGTH: 18  
 <212> TYPE: PRT  
 <213> ORGANISM: Artificial Sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: Description of Artificial Sequence:antibody  
 198/A1 derived mutant peptide A1/3-30 Glutamic acid scan W-9-E-9

&lt;400&gt; SEQUENCE: 39

Arg Arg Arg Glu Gly Gly Tyr Tyr Val Glu Trp Tyr Phe Asp Arg  
 1 5 10 15

Arg Arg

<210> SEQ ID NO 40  
 <211> LENGTH: 18  
 <212> TYPE: PRT  
 <213> ORGANISM: Artificial Sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: Description of Artificial Sequence:antibody  
 198/A1 derived mutant peptide A1/3-31 Glutamic acid scan Y-10-E-10

&lt;400&gt; SEQUENCE: 40

Arg Arg Arg Glu Gly Gly Tyr Tyr Val Asn Trp Glu Phe Asp Arg  
 1 5 10 15

Arg Arg

<210> SEQ ID NO 41  
 <211> LENGTH: 18  
 <212> TYPE: PRT  
 <213> ORGANISM: Artificial Sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: Description of Artificial Sequence:antibody  
 198/A1 derived mutant peptide A1/3-32 Glutamic acid scan F-11-E-11

&lt;400&gt; SEQUENCE: 41

Arg Arg Arg Glu Gly Gly Tyr Tyr Val Asn Trp Tyr Glu Asp Arg  
 1 5 10 15

Arg Arg

<210> SEQ ID NO 42  
 <211> LENGTH: 18  
 <212> TYPE: PRT  
 <213> ORGANISM: Artificial Sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: Description of Artificial Sequence:antibody  
 198/A1 derived mutant peptide A1/3-33 Glutamic acid scan D12-E-12

&lt;400&gt; SEQUENCE: 42

Arg Arg Arg Glu Gly Gly Tyr Tyr Val Asn Trp Tyr Phe Glu Arg  
 1 5 10 15

Arg Arg

<210> SEQ ID NO 43  
 <211> LENGTH: 18  
 <212> TYPE: PRT  
 <213> ORGANISM: Artificial Sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: Description of Artificial Sequence:antibody  
 198/A1 derived mutant peptide A1/3-34srmb scrambled version

&lt;400&gt; SEQUENCE: 43

Arg Arg Arg Gly Glu Tyr Gly Glu Tyr Trp Asn Gly Asp Phe Tyr Arg  
 1 5 10 15

US 7,033,590 B1

51

52

-continued

Arg Arg

&lt;210&gt; SEQ ID NO 44

&lt;400&gt; SEQUENCE: 44

000

&lt;210&gt; SEQ ID NO 45

&lt;211&gt; LENGTH: 14

&lt;212&gt; TYPE: PRT

&lt;213&gt; ORGANISM: Artificial Sequence

&lt;220&gt; FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence:antibody  
198/B1 derived mutated peptide B1/4

&lt;400&gt; SEQUENCE: 45

Arg Glu Gly Gly Gly Phe Thr Val Asn Trp Tyr Phe Asp Arg  
1 5 10

&lt;210&gt; SEQ ID NO 46

&lt;211&gt; LENGTH: 14

&lt;212&gt; TYPE: PRT

&lt;213&gt; ORGANISM: Artificial Sequence

&lt;220&gt; FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence:antibody  
198/B1 derived mutated peptide B1/5 scrambled version

&lt;400&gt; SEQUENCE: 46

Phe Gly Val Gly Tyr Arg Gly Glu Thr Arg Asn Phe Asp Trp  
1 5 10

&lt;210&gt; SEQ ID NO 47

&lt;211&gt; LENGTH: 18

&lt;212&gt; TYPE: PRT

&lt;213&gt; ORGANISM: Artificial Sequence

&lt;220&gt; FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence:antibody  
198/B1 derived mutated peptide B1/6

&lt;400&gt; SEQUENCE: 47

Glu Glu Glu Glu Gly Gly Gly Phe Thr Val Asn Trp Tyr Phe Asp Glu  
1 5 10 15

Glu Glu

&lt;210&gt; SEQ ID NO 48

&lt;211&gt; LENGTH: 18

&lt;212&gt; TYPE: PRT

&lt;213&gt; ORGANISM: Artificial Sequence

&lt;220&gt; FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence:antibody  
198/B1 derived mutated peptide B1/7

&lt;400&gt; SEQUENCE: 48

Arg Arg Arg Glu Gly Gly Gly Phe Thr Val Asn Trp Tyr Phe Asp Arg  
1 5 10 15

Arg Arg

&lt;210&gt; SEQ ID NO 49

&lt;211&gt; LENGTH: 18

&lt;212&gt; TYPE: PRT

&lt;213&gt; ORGANISM: Artificial Sequence

&lt;220&gt; FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence:antibody  
198/B1 derived mutated peptide B1/7scr3 scrambled version

US 7,033,590 B1

53

54

-continued

&lt;400&gt; SEQUENCE: 49

Arg Arg Arg Phe Gly Val Gly Tyr Gly Glu Thr Asn Phe Asp Trp Arg  
 1 5 10 15

Arg Arg

&lt;210&gt; SEQ ID NO 50

&lt;211&gt; LENGTH: 57

&lt;212&gt; TYPE: DNA

&lt;213&gt; ORGANISM: Artificial Sequence

&lt;220&gt; FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence:mouse V-H  
back primer VH1BACK-Sfi

&lt;400&gt; SEQUENCE: 50

catgccatga ctgcggcccc agccggccat ggccsaggtg marctgcags agtcwgg 57

&lt;210&gt; SEQ ID NO 51

&lt;211&gt; LENGTH: 56

&lt;212&gt; TYPE: DNA

&lt;213&gt; ORGANISM: Artificial Sequence

&lt;220&gt; FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence:mouse V-H  
back primer VH1BACKSfi

&lt;400&gt; SEQUENCE: 51

gtcctcgcaa ctgcggccca gccggccatg gccgaggtgc agcttcagga gtcagg 56

&lt;210&gt; SEQ ID NO 52

&lt;211&gt; LENGTH: 56

&lt;212&gt; TYPE: DNA

&lt;213&gt; ORGANISM: Artificial Sequence

&lt;220&gt; FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence:mouse V-H  
back primer VH2BACKSfi

&lt;400&gt; SEQUENCE: 52

gtcctcgcaa ctgcggccca gccggccatg gccgatgtgc agcttcagga gtcagg 56

&lt;210&gt; SEQ ID NO 53

&lt;211&gt; LENGTH: 56

&lt;212&gt; TYPE: DNA

&lt;213&gt; ORGANISM: Artificial Sequence

&lt;220&gt; FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence:mouse V-H  
back primer VH3BACKSfi

&lt;400&gt; SEQUENCE: 53

gtcctcgcaa ctgcggccca gccggccatg gccaggtgc agctgaagsa gtcagg 56

&lt;210&gt; SEQ ID NO 54

&lt;211&gt; LENGTH: 56

&lt;212&gt; TYPE: DNA

&lt;213&gt; ORGANISM: Artificial Sequence

&lt;220&gt; FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence:mouse V-H  
back primer VH4/6BACKSfi

&lt;400&gt; SEQUENCE: 54

gtcctcgcaa ctgcggccca gccggccatg gccgaggtgc agctgcarga rtctgg 56

&lt;210&gt; SEQ ID NO 55

&lt;211&gt; LENGTH: 56

&lt;212&gt; TYPE: DNA

&lt;213&gt; ORGANISM: Artificial Sequence

&lt;220&gt; FEATURE:

&lt;223&gt; OTHER INFORMATION: Description of Artificial Sequence:mouse V-H

US 7,033,590 B1

55

56

-continued

---

back primer VH5/9BACKSfi

<400> SEQUENCE: 55

gtcctcgcaa ctgcggccca gccggccatg gccaggtgc arctgcagca gytctg 56

<210> SEQ ID NO 56  
 <211> LENGTH: 56  
 <212> TYPE: DNA  
 <213> ORGANISM: Artificial Sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: Description of Artificial Sequence:mouse V-H  
 back primer VH7BACKSfi

<400> SEQUENCE: 56

gtcctcgcaa ctgcggccca gccggccatg gccgargtga agctggtgga rtctgg 56

<210> SEQ ID NO 57  
 <211> LENGTH: 56  
 <212> TYPE: DNA  
 <213> ORGANISM: Artificial Sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: Description of Artificial Sequence:mouse V-H  
 back primer VH8BACKSfi

<400> SEQUENCE: 57

gtcctcgcaa ctgcggccca gccggccatg gccgaggttc agcttcagca gtctgg 56

<210> SEQ ID NO 58  
 <211> LENGTH: 56  
 <212> TYPE: DNA  
 <213> ORGANISM: Artificial Sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: Description of Artificial Sequence:mouse V-H  
 back primer VH10BACKSfi

<400> SEQUENCE: 58

gtcctcgcaa ctgcggccca gccggccatg gccgaagtgc agctgktgga gwtctg 56

<210> SEQ ID NO 59  
 <211> LENGTH: 56  
 <212> TYPE: DNA  
 <213> ORGANISM: Artificial Sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: Description of Artificial Sequence:mouse V-H  
 back primer VH11BACKSfi

<400> SEQUENCE: 59

gtcctcgcaa ctgcggccca gccggccatg gccagatcc agttgctgca gtctgg 56

<210> SEQ ID NO 60  
 <211> LENGTH: 68  
 <212> TYPE: DNA  
 <213> ORGANISM: Artificial Sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: Description of Artificial Sequence:mouse J-H  
 forward primer VH1FOR2LiAsc

<400> SEQUENCE: 60

accgccagag gcgcgcccac ctgaaccgcc tccacctgag gagacggtga ccgtggtecc 60

ttggcccc 68

<210> SEQ ID NO 61  
 <211> LENGTH: 60  
 <212> TYPE: DNA  
 <213> ORGANISM: Artificial Sequence  
 <220> FEATURE:

US 7,033,590 B1

57

58

-continued

---

```

<223> OTHER INFORMATION: Description of Artificial Sequence:mouse J-H
forward primer JH1FORLiAsc

<400> SEQUENCE: 61

accgccagag gcgcgcccac ctgaaccgcc tccacctgag gagacggtga ccgtgggtccc      60

<210> SEQ ID NO 62
<211> LENGTH: 60
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:mouse J-H
forward primer JH2FORLiAsc

<400> SEQUENCE: 62

accgccagag gcgcgcccac ctgaaccgcc tccacctgag gagactgtga gagtgggtgcc      60

<210> SEQ ID NO 63
<211> LENGTH: 60
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:mouse J-H
forward primer JH3FORLiAsc

<400> SEQUENCE: 63

accgccagag gcgcgcccac ctgaaccgcc tccacctgca gagacagtga ccagagtccc      60

<210> SEQ ID NO 64
<211> LENGTH: 60
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:mouse J-H
forward primer JH4FORLiAsc

<400> SEQUENCE: 64

accgccagag gcgcgcccac ctgaaccgcc tccacctgag gagacggtga ctgagggttcc      60

<210> SEQ ID NO 65
<211> LENGTH: 60
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:mouse
V-kappa back primer VK2BACK-LiAscI

<400> SEQUENCE: 65

ggttcagatg ggcgcgcctc tggcgggtggc ggatcggaca ttgagctcac ccagtctcca      60

<210> SEQ ID NO 66
<211> LENGTH: 59
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:mouse
V-kappa back primer VK1BACKLi Asc

<400> SEQUENCE: 66

ggttcagatg ggcgcgcctc tggcgggtggc ggatcggaca ttgtgatgwc acagtctcc      59

<210> SEQ ID NO 67
<211> LENGTH: 59
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:mouse

```



US 7,033,590 B1

59

60

-continued

---

V-kappa back primer VK2BACKLi Asc

<400> SEQUENCE: 67

ggttcagatg ggcgcgcctc tggcgggtggc ggatcggatg tktgatgac ccaaactcc 59

<210> SEQ ID NO 68  
 <211> LENGTH: 59  
 <212> TYPE: DNA  
 <213> ORGANISM: Artificial Sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: Description of Artificial Sequence:mouse  
 V-kappa back primer VK3BACKLi Asc

<400> SEQUENCE: 68

ggttcagatg ggcgcgcctc tggcgggtggc ggatcggata ttgtgatrac bcaggcwgcc 59

<210> SEQ ID NO 69  
 <211> LENGTH: 59  
 <212> TYPE: DNA  
 <213> ORGANISM: Artificial Sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: Description of Artificial Sequence:mouse  
 V-kappa back primer VK4BACKLi Asc

<400> SEQUENCE: 69

ggttcagatg ggcgcgcctc tggcgggtggc ggatcggaca ttgtgctgac mcartctcc 59

<210> SEQ ID NO 70  
 <211> LENGTH: 59  
 <212> TYPE: DNA  
 <213> ORGANISM: Artificial Sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: Description of Artificial Sequence:mouse  
 V-kappa back primer VK5BACKLi Asc

<400> SEQUENCE: 70

ggttcagatg ggcgcgcctc tggcgggtggc ggatcgsaaa wtgtkctcac ccagtctcc 59

<210> SEQ ID NO 71  
 <211> LENGTH: 59  
 <212> TYPE: DNA  
 <213> ORGANISM: Artificial Sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: Description of Artificial Sequence:mouse  
 V-kappa back primer VK6BACKLi Asc

<400> SEQUENCE: 71

ggttcagatg ggcgcgcctc tggcgggtggc ggatcggaya tyvwgatgac mcagwtccc 59

<210> SEQ ID NO 72  
 <211> LENGTH: 59  
 <212> TYPE: DNA  
 <213> ORGANISM: Artificial Sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: Description of Artificial Sequence:mouse  
 V-kappa back primer VK7BACKLi Asc

<400> SEQUENCE: 72

ggttcagatg ggcgcgcctc tggcgggtggc ggatcgaaa ttgttctcac ccagtctcc 59

<210> SEQ ID NO 73  
 <211> LENGTH: 59  
 <212> TYPE: DNA  
 <213> ORGANISM: Artificial Sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: Description of Artificial Sequence:mouse  
 V-kappa back primer VK8BACKLi Asc

US 7,033,590 B1

61

62

-continued

---

```

<400> SEQUENCE: 73
gggtcagatg ggcgcgcctc tggcgggtggc ggatcgatcat tattgcaggt gcttggtgg 59

<210> SEQ ID NO 74
<211> LENGTH: 42
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:mouse
      J-kappa forward primer JK1NOT10

<400> SEQUENCE: 74
gagtcattct gcggccgccc gtttgatttc cagcttggtg cc 42

<210> SEQ ID NO 75
<211> LENGTH: 42
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:mouse
      J-kappa forward primer JK2NOT10

<400> SEQUENCE: 75
gagtcattct gcggccgccc gttttatttc cagcttggtc cc 42

<210> SEQ ID NO 76
<211> LENGTH: 42
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:mouse
      J-kappa forward primer JK3NOT10

<400> SEQUENCE: 76
gagtcattct gcggccgccc gttttatttc cagtctggtc cc 42

<210> SEQ ID NO 77
<211> LENGTH: 42
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:mouse
      J-kappa forward primer JK4NOT10

<400> SEQUENCE: 77
gagtcattct gcggccgccc gttttatttc caactttggtc cc 42

<210> SEQ ID NO 78
<211> LENGTH: 42
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:mouse
      J-kappa forward primer JK5NOT10

<400> SEQUENCE: 78
gagtcattct gcggccgccc gtttcagctc cagcttggtc cc 42

<210> SEQ ID NO 79
<211> LENGTH: 74
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:
      oligonucleotide mychis6-co

```

US 7,033,590 B1

63

64

-continued

&lt;400&gt; SEQUENCE: 79

ggccgcagaa caaaaactca tctcagaaga ggatctgaat ggggcggcac atcaccatca 60

ccatcactaa taag 74

&lt;210&gt; SEQ ID NO 80

&lt;211&gt; LENGTH: 74

&lt;212&gt; TYPE: DNA

&lt;213&gt; ORGANISM: Artificial Sequence

&lt;220&gt; FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence:  
oligonucleotide mycchis-ic

&lt;400&gt; SEQUENCE: 80

aattcttatt agtggatggtg atggatgatgt gccgcccac tcagatcctc ttctgagatg 60

agtttttgtt ctgc 74

&lt;210&gt; SEQ ID NO 81

&lt;211&gt; LENGTH: 726

&lt;212&gt; TYPE: DNA

&lt;213&gt; ORGANISM: Artificial Sequence

&lt;220&gt; FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence:scFv from  
hybridoma cell line 193/AD3

&lt;400&gt; SEQUENCE: 81

gaggtgaagc tgggtggagtc tggacctgag ctgaagaagc ctggagagac agtcaagatc 60

tctctgaagg cttctgggta tatcttcaca aactatggaa tgaactgggt gaagcaggct 120

ccaggaaagg gtttaagtg gatgggctgg ataaacacot acactggaga gccaacatat 180

gctgatgact tcaagggaagc gtttgccctc tctttggaaa cctctgccag cactgcctat 240

ttgcagatca acaacctcaa aaatgaggac acggctacat atttctgtgc attatatggt 300

aactccccta aggggttttc ttactggggc caagggaactc tggtcactgt ctctgcagggt 360

ggaggcgggt cagggtggcg cgcctctggc ggtggcggat cggatattca gatgacacag 420

tctcccaaat tctgcttgt atcagcagga gacagggtta ccataacctg caaggccagt 480

cagagtgtga gtaatgatgt agcttggtac caacagaagc cggggcagtc tcctaaacta 540

ctgatgtact atgcatccaa tcgctacact ggagtcctcg atcgcttcac tggcagtggg 600

tatgggacgg atttcacttt caccatcagc actgtgcagg ctgaagacct ggcagtttat 660

ttctgtcagc aggattatgg ctctcctccc acgttcggag ggggcaccaa gctggaaatt 720

aaacgg 726

&lt;210&gt; SEQ ID NO 82

&lt;211&gt; LENGTH: 242

&lt;212&gt; TYPE: PRT

&lt;213&gt; ORGANISM: Artificial Sequence

&lt;220&gt; FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence:scFv from  
hybridoma cell line 193/AD3

&lt;400&gt; SEQUENCE: 82

Glu Val Lys Leu Val Glu Ser Gly Pro Glu Leu Lys Lys Pro Gly Glu  
1 5 10 15Thr Val Lys Ile Ser Cys Lys Ala Ser Gly Tyr Ile Phe Thr Asn Tyr  
20 25 30Gly Met Asn Trp Val Lys Gln Ala Pro Gly Lys Gly Leu Lys Trp Met  
35 40 45

Gly Trp Ile Asn Thr Tyr Thr Gly Glu Pro Thr Tyr Ala Asp Asp Phe

US 7,033,590 B1

65

66

-continued

50	55	60
Lys Gly Arg Phe Ala Phe Ser Leu Glu Thr Ser Ala Ser Thr Ala Tyr		
65	70	75 80
Leu Gln Ile Asn Asn Leu Lys Asn Glu Asp Thr Ala Thr Tyr Phe Cys		
	85	90 95
Ala Leu Tyr Gly Asn Ser Pro Lys Gly Phe Ala Tyr Trp Gly Gln Gly		
	100	105 110
Thr Leu Val Thr Val Ser Ala Gly Gly Gly Gly Ser Gly Gly Arg Ala		
	115	120 125
Ser Gly Gly Gly Gly Ser Asp Ile Gln Met Thr Gln Ser Pro Lys Phe		
	130	135 140
Leu Leu Val Ser Ala Gly Asp Arg Val Thr Ile Thr Cys Lys Ala Ser		
	145	150 155 160
Gln Ser Val Ser Asn Asp Val Ala Trp Tyr Gln Gln Lys Pro Gly Gln		
	165	170 175
Ser Pro Lys Leu Leu Met Tyr Tyr Ala Ser Asn Arg Tyr Thr Gly Val		
	180	185 190
Pro Asp Arg Phe Thr Gly Ser Gly Tyr Gly Thr Asp Phe Thr Phe Thr		
	195	200 205
Ile Ser Thr Val Gln Ala Glu Asp Leu Ala Val Tyr Phe Cys Gln Gln		
	210	215 220
Asp Tyr Gly Ser Pro Pro Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile		
	225	230 235 240
Lys Arg		

<210> SEQ ID NO 83  
 <211> LENGTH: 747  
 <212> TYPE: DNA  
 <213> ORGANISM: Artificial Sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: Description of Artificial Sequence: scFv from  
 hybridoma cell line 193/K2

<400> SEQUENCE: 83

gaagtcgacg tgggtggagtc tgggggaggc ctagtgaagc ctggagggtc cctgaaactc	60
tcctgtgcag cctctggatt cactttcagt acctatacca tgtcttgggt tcgccagact	120
cggagaaga ggctggagtg ggtcgcaacc attagtagtg gtggtagtta caccctactat	180
ccagacagtg tgaggggccc attcaccatc tccagagaca atgccaagaa caccctgtac	240
ctgcaaatga gcagctctgaa gtctgaggac acagccatgt attactgtac aagagatggg	300
ggacacgggt acggtagtag ctttgactac tggggccaag gcaccactct cacagtctcc	360
tcaggtggag gcggttcagg tgggcgcgcc tctggcgggt gcggatcgca aattgtgctc	420
accagtcctc cactctccct gcctgtcagt cttggagatc aagcctccat ctcttgcaga	480
tctagtcaga gcattgtaca tagtaatgga aacacctatt tagaatggta cctgcagaaa	540
ccaggccagt ctccaaagct cctgatctac aaagtttcca accgattttc tgggggtccca	600
gacaaattca gtggcagtggt atcagggaca gatttcacac tcaagatcag cagagtggag	660
gctgaggatc tgggagttta ttactgcttt caaggttcac atgttccgtg gacgttcggt	720
ggaggcacca agctggaaat caaacgg	747

<210> SEQ ID NO 84  
 <211> LENGTH: 249  
 <212> TYPE: PRT  
 <213> ORGANISM: Artificial Sequence

US 7,033,590 B1

67

68

-continued

&lt;220&gt; FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence:scFv from  
hybridoma cell line 193/K2

&lt;400&gt; SEQUENCE: 84

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Lys Pro Gly Gly  
 1 5 10 15  
 Ser Leu Lys Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Thr Tyr  
 20 25 30  
 Thr Met Ser Trp Val Arg Gln Thr Pro Glu Lys Arg Leu Glu Trp Val  
 35 40 45  
 Ala Thr Ile Ser Ser Gly Gly Ser Tyr Thr Tyr Tyr Pro Asp Ser Val  
 50 55 60  
 Arg Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Leu Tyr  
 65 70 75 80  
 Leu Gln Met Ser Ser Leu Lys Ser Glu Asp Thr Ala Met Tyr Tyr Cys  
 85 90 95  
 Thr Arg Asp Gly Gly His Gly Tyr Gly Ser Ser Phe Asp Tyr Trp Gly  
 100 105 110  
 Gln Gly Thr Thr Leu Thr Val Ser Ser Gly Gly Gly Gly Ser Gly Gly  
 115 120 125  
 Arg Ala Ser Gly Gly Gly Gly Ser Gln Ile Val Leu Thr Gln Ser Pro  
 130 135 140  
 Leu Ser Leu Pro Val Ser Leu Gly Asp Gln Ala Ser Ile Ser Cys Arg  
 145 150 155 160  
 Ser Ser Gln Ser Ile Val His Ser Asn Gly Asn Thr Tyr Leu Glu Trp  
 165 170 175  
 Tyr Leu Gln Lys Pro Gly Gln Ser Pro Lys Leu Leu Ile Tyr Lys Val  
 180 185 190  
 Ser Asn Arg Phe Ser Gly Val Pro Asp Lys Phe Ser Gly Ser Gly Ser  
 195 200 205  
 Gly Thr Asp Phe Thr Leu Lys Ile Ser Arg Val Glu Ala Glu Asp Leu  
 210 215 220  
 Gly Val Tyr Tyr Cys Phe Gln Gly Ser His Val Pro Trp Thr Phe Gly  
 225 230 235 240  
 Gly Gly Thr Lys Leu Glu Ile Lys Arg  
 245

&lt;210&gt; SEQ ID NO 85

&lt;211&gt; LENGTH: 747

&lt;212&gt; TYPE: DNA

&lt;213&gt; ORGANISM: Artificial Sequence

&lt;220&gt; FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence:scFv from  
hybridoma cell line 198/AB2 (subclone of 198/B1)

&lt;400&gt; SEQUENCE: 85

gaggtgcagc ttcaggagtc agggggaggc ttagtgaagc ctggagggtc cctgaaactc 60  
 tcctgtgcag cctctggatt cactttcagt agctatacca tgtcttggtt tcgccagact 120  
 ccggagaaga ggctggagtg ggtcgcaacc attagtagtg gtggtagtac caccctactat 180  
 ccagacagtg tgaagggccg attcaccatc tccagagaca atgccaagaa caccctgtac 240  
 ctgcaaatga gcagtctgag gtctgaggac acagccatgt attactgtac aagagagggg 300  
 ggtgttttca ccgtcaactg gtacttcgat gtctggggcg cagggactct ggtcactgtc 360  
 tctgcaggtg gaggcgggtc aggtggggcg gcctctggcg gtggcggatc ggaaatgtg 420

US 7,033,590 B1

69

70

-continued

---

```

ctcaccocagt ctccagcttc tttggctgtg tctctagggc agagggccac catatcctgc 480
agagccagtg aaagtgttga tagttatggc tataatttta tgcactggta tcagcagata 540
ccaggacagc caccctaaact cctcatctat cgtgcatcca acctagagtc tgggatccct 600
gccaggttca gtggcagtggt gtctaggaca gacttcaccc tcaccattaa tcctgtggag 660
gctgatgatg ttgcaaccta ttactgtcag caaagtaatg aggatccgct caggttcggt 720
actgggacca gactggaaat aaaacgg 747

```

```

<210> SEQ ID NO 86
<211> LENGTH: 249
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:scFv from
hybridoma cell line 198/AB2 (subclone of 198/B1)

```

```

<400> SEQUENCE: 86

```

```

Glu Val Gln Leu Gln Glu Ser Gly Gly Gly Leu Val Lys Pro Gly Gly
1          5          10          15
Ser Leu Lys Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
20          25          30
Thr Met Ser Trp Val Arg Gln Thr Pro Glu Lys Arg Leu Glu Trp Val
35          40          45
Ala Thr Ile Ser Ser Gly Gly Ser Ser Thr Tyr Tyr Pro Asp Ser Val
50          55          60
Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Leu Tyr
65          70          75          80
Leu Gln Met Ser Ser Leu Arg Ser Glu Asp Thr Ala Met Tyr Tyr Cys
85          90          95
Thr Arg Glu Gly Gly Gly Phe Thr Val Asn Trp Tyr Phe Asp Val Trp
100         105         110
Gly Ala Gly Thr Leu Val Thr Val Ser Ala Gly Gly Gly Ser Gly
115         120         125
Gly Arg Ala Ser Gly Gly Gly Gly Ser Glu Asn Val Leu Thr Gln Ser
130         135         140
Pro Ala Ser Leu Ala Val Ser Leu Gly Gln Arg Ala Thr Ile Ser Cys
145         150         155         160
Arg Ala Ser Glu Ser Val Asp Ser Tyr Gly Tyr Asn Phe Met His Trp
165         170         175
Tyr Gln Gln Ile Pro Gly Gln Pro Pro Lys Leu Leu Ile Tyr Arg Ala
180         185         190
Ser Asn Leu Glu Ser Gly Ile Pro Ala Arg Phe Ser Gly Ser Gly Ser
195         200         205
Arg Thr Asp Phe Thr Leu Thr Ile Asn Pro Val Glu Ala Asp Asp Val
210         215         220
Ala Thr Tyr Tyr Cys Gln Gln Ser Asn Glu Asp Pro Leu Thr Phe Gly
225         230         235         240
Thr Gly Thr Arg Leu Glu Ile Lys Arg
245

```

```

<210> SEQ ID NO 87
<211> LENGTH: 747
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:scFv derived
from hybridoma cell line 198/A1

```

US 7,033,590 B1

71

72

-continued

---

```

<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(747)
<223> OTHER INFORMATION: n = g, a, c or t

<400> SEQUENCE: 87

gaggtgcagc ttcaggagtc agggggaggc ttagtgaagc ctggagggtc cctgaaactc      60
tcctgtgcag cctctggatt catttttagt agttatacca tgtcttgggt tcgccagact      120
ccggagaaga ggctggagtg ggtcgcaacc attagtagtg gtggtagttc cacctactat      180
ccagacagtg tgaagggccg attcaccatc tccagagaca atgccaagaa caccctgtac      240
ctgcaaatga gcagtctgaa gtctgaggac acagccatgt atcactgtac aagagagggg      300
ggtggttatt acgtcaactg gtacttcgat gtctggggcg caggcaccac tctcacagtc      360
tcctcaggtg gaggcgggtc aggtggggcg gcctctggcg gtggcggatc ggacattgag      420
ctcacncagt ctccagcttc tttggctgtg tctctagggc agagggccac catatcctgc      480
agagccagtg aaagtgttga tagttatggc aagagtttta tgcaactgta ccagcagaaa      540
ccagggcagc caccctaaat cctcatctat cgtgcacca acctagaatc tgggatccct      600
gccaggttca gtggcagtggt gtctaggaca gacttcaccc tcaccattaa tcctgtggag      660
gctgatgatg ttgcnaccta ttactgtcag caaagtaatg aggatccctc cagttcgggt      720
gctgggacca gactggaaat aaaacgg                                     747

```

```

<210> SEQ ID NO 88
<211> LENGTH: 249
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: scFv derived
from hybridoma cell line 198/A1

```

```

<400> SEQUENCE: 88

Glu Val Gln Leu Gln Glu Ser Gly Gly Gly Leu Val Lys Pro Gly Gly
 1           5           10           15

Ser Leu Lys Leu Ser Cys Ala Ala Ser Gly Phe Ile Phe Ser Ser Tyr
      20           25           30

Thr Met Ser Trp Val Arg Gln Thr Pro Glu Lys Arg Leu Glu Trp Val
      35           40           45

Ala Thr Ile Ser Ser Gly Gly Ser Ser Thr Tyr Tyr Pro Asp Ser Val
      50           55           60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Leu Tyr
      65           70           75           80

Leu Gln Met Ser Ser Leu Lys Ser Glu Asp Thr Ala Met Tyr His Cys
      85           90           95

Thr Arg Glu Gly Gly Gly Tyr Tyr Val Asn Trp Tyr Phe Asp Val Trp
      100          105          110

Gly Ala Gly Thr Thr Leu Thr Val Ser Ser Gly Gly Gly Gly Ser Gly
      115          120          125

Gly Arg Ala Ser Gly Gly Gly Gly Ser Asp Ile Glu Leu Thr Gln Ser
      130          135          140

Pro Ala Ser Leu Ala Val Ser Leu Gly Gln Arg Ala Thr Ile Ser Cys
      145          150          155          160

Arg Ala Ser Glu Ser Val Asp Ser Tyr Gly Lys Ser Phe Met His Trp
      165          170          175

Tyr Gln Gln Lys Pro Gly Gln Pro Pro Lys Leu Leu Ile Tyr Arg Ala
      180          185          190

```

US 7,033,590 B1

73

74

-continued

Ser Asn Leu Glu Ser Gly Ile Pro Ala Arg Phe Ser Gly Ser Gly Ser  
195 200 205

Arg Thr Asp Phe Thr Leu Thr Ile Asn Pro Val Glu Ala Asp Asp Val  
210 215 220

Ala Thr Tyr Tyr Cys Gln Gln Ser Asn Glu Asp Pro Leu Thr Phe Gly  
225 230 235 240

Ala Gly Thr Arg Leu Glu Ile Lys Arg  
245

<210> SEQ ID NO 89  
<211> LENGTH: 2199  
<212> TYPE: DNA  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Description of Artificial Sequence:198A2  
scFv-alkaline phosphatase fusion protein (ORF of expression  
vector pDAP2-198AB2#100)  
<220> FEATURE:  
<221> NAME/KEY: modified\_base  
<222> LOCATION: (228)  
<223> OTHER INFORMATION: n = g, a, c or t  
  
<400> SEQUENCE: 89

```

atgaaatacc tattgcctac ggcagccgct ggattgttat tactcgcgcc ccagccggcc 60
atggcggagg tgaagctggt ggagctctgg ggaggcttag tgaagcctgg agggctccctg 120
aaactctcct gtgcagcctc tggattcact ttcagtagct ataccatgtc ttgggttcgc 180
cagactcccg agaagaggct ggagtgggtc gcaaccatta gtagtggnng tagttccacc 240
tactatccag acagtgtgaa gggccgattc accatctcca gagacaatgc caagaacacc 300
ctgtacctgc aaatgagcag tctgaggtct gaggacacag ccatgtatta ctgtacaaga 360
gaggggggtg gtttcaccgt caactggtag ttcgatgtct gggcgcgagg aaacctcagtc 420
accgtctcct caggtggagg cggttcaggt gggcgcgccct ctggcggtgg cggtatcgac 480
attgtgctga cacagtctcc agcttctttg gctgtgtctc tagggcagag gccaccata 540
tcctgcagag ccagtgaag tgttgatagt tatggctata attttatgca ctggtatcag 600
cagataccag gacagccacc caaactcctc atctatcgtg catccaacct agagtctggg 660
atccctgcca ggttcagtgg cagtgggtct aggacagact tcaccctcac cattaatcct 720
gtggaggctg atgatgttg aacctattac tgtcagcaaa gtaatgagga tccgctcacg 780
ttcgggtactg ggaccagact ggaaataaaa cggcgggcgg cagcccgggc accagaaatg 840
cctgttcttg aaaaccgggc tgctcagggc gatattactg cacccgcgcg tgctcgccgt 900
ttaacgggtg atcagactcg cgctctgcgt gattctctta gcgataaacc tgcaaaaaat 960
attatttttg tgattggcga tgggtagggg gactcggaaa ttactgccgc acgtaattat 1020
gccgaagggt cggggcggtt ttttaaaggt atagatgcct taccgcttac cgggcaatac 1080
actcactatg cgctgaataa aaaaaccggc aaaccggact acgtcaccga ctcggtgca 1140
tcagcaaccg cctggtcaac cggtgtcaaa acctataacg gcgcgctggg cgtcgatatt 1200
cacgaaaaag atcacccaac gattctggaa atggcaaaag ccgcaggtct ggcgaccggt 1260
aacgtttcta ccgcagagtt gcaggatgcc acgcccgtcg cgctggtggc acatgtgacc 1320
tcgcgcaaat gctacggctc gagcgcgacc agtgaaaaat gtcgggttaa cgctctggaa 1380
aaaggcgcaa aaggatcgat taccgaacag ctgcttaacg ctgctgccga cgttacgctt 1440
ggcgggcgcg caaaaacctt tgctgaaacg gcaaccgctg gtgaatggca gggaaaaacg 1500

```



US 7,033,590 B1

75

76

-continued

---

```

ctgcgtgaac aggcacagcg gcgtggttat cagttggtga gcgatgctgc ctoactgaat 1560
tcggtgacgg aagcgaatca gcaaaaacccc ctgcttgccc tgtttgctga cggaatatg 1620
ccagtgcgct ggctaggacc gaaagcaacg taccatggca atatcgataa gcccgagtc 1680
acctgtacgc caaatccgca acgtaatgac agtgtaccaa ccctggcgca gatgaccgac 1740
aaagccattg aattgttgag taaaaatgag aaaggctttt tcctgcaagt tgaagggtcg 1800
tcaatcgata aacaggatca tgctgcgaat ccttggtggc aaattggcga gacggtcgat 1860
ctcgatgaag ccgtacaacg ggcgctggaa ttcgctaaaa aggagggtaa cacgctggtc 1920
atagtccacg ctgatcacgc ccacgccagc cagattgttg cgccggatac caaagctccg 1980
ggcctcaccg aggcgctaaa taccaaagat ggcgcagtga tggatgatgag ttacgggaac 2040
tccgaagagg attcacaaga acataccggc agtcagttgc gtattgcggc gtatggcccg 2100
catgccgcca atgttggttg actgaccgac cagaccgatc tcttctacac catgaaagcc 2160
gctctggggg atatcgcaac ccatcaccat caccattaa 2199

```

```

<210> SEQ ID NO 90
<211> LENGTH: 732
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:198A2
scFv-alkaline phosphatase fusion protein (ORF of expression
vector pDAP2-198AB2#100)

```

```

<400> SEQUENCE: 90

```

```

Met Lys Tyr Leu Leu Pro Thr Ala Ala Ala Gly Leu Leu Leu Leu Ala
 1             5             10             15
Ala Gln Pro Ala Met Ala Glu Val Lys Leu Val Glu Ser Gly Gly Gly
 20             25             30
Leu Val Lys Pro Gly Gly Ser Leu Lys Leu Ser Cys Ala Ala Ser Gly
 35             40             45
Phe Thr Phe Ser Ser Tyr Thr Met Ser Trp Val Arg Gln Thr Pro Glu
 50             55             60
Lys Arg Leu Glu Trp Val Ala Thr Ile Ser Ser Gly Gly Ser Ser Thr
 65             70             75             80
Tyr Tyr Pro Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn
 85             90             95
Ala Lys Asn Thr Leu Tyr Leu Gln Met Ser Ser Leu Arg Ser Glu Asp
 100            105            110
Thr Ala Met Tyr Tyr Cys Thr Arg Glu Gly Gly Gly Phe Thr Val Asn
 115            120            125
Trp Tyr Phe Asp Val Trp Gly Ala Gly Thr Ser Val Thr Val Ser Ser
 130            135            140
Gly Gly Gly Gly Ser Gly Gly Arg Ala Ser Gly Gly Gly Gly Ser Asp
 145            150            155            160
Ile Val Leu Thr Gln Ser Pro Ala Ser Leu Ala Val Ser Leu Gly Gln
 165            170            175
Arg Ala Thr Ile Ser Cys Arg Ala Ser Glu Ser Val Asp Ser Tyr Gly
 180            185            190
Tyr Asn Phe Met His Trp Tyr Gln Gln Ile Pro Gly Gln Pro Pro Lys
 195            200            205
Leu Leu Ile Tyr Arg Ala Ser Asn Leu Glu Ser Gly Ile Pro Ala Arg
 210            215            220
Phe Ser Gly Ser Gly Ser Arg Thr Asp Phe Thr Leu Thr Ile Asn Pro

```

US 7,033,590 B1

77

78

-continued

225	230	235	240
Val Glu Ala Asp Asp	Val Ala Thr Tyr Tyr	Cys Gln Gln Ser Asn Glu	
	245	250	255
Asp Pro Leu Thr Phe Gly Thr Gly Thr Arg Leu Glu Ile Lys Arg Ala			
	260	265	270
Ala Ala Ala Arg Ala Pro Glu Met Pro Val Leu Glu Asn Arg Ala Ala			
	275	280	285
Gln Gly Asp Ile Thr Ala Pro Gly Gly Ala Arg Arg Leu Thr Gly Asp			
	290	295	300
Gln Thr Ala Ala Leu Arg Asp Ser Leu Ser Asp Lys Pro Ala Lys Asn			
	305	310	315
Ile Ile Leu Leu Ile Gly Asp Gly Met Gly Asp Ser Glu Ile Thr Ala			
	325	330	335
Ala Arg Asn Tyr Ala Glu Gly Ala Gly Phe Phe Lys Gly Ile Asp			
	340	345	350
Ala Leu Pro Leu Thr Gly Gln Tyr Thr His Tyr Ala Leu Asn Lys Lys			
	355	360	365
Thr Gly Lys Pro Asp Tyr Val Thr Asp Ser Ala Ala Ser Ala Thr Ala			
	370	375	380
Trp Ser Thr Gly Val Lys Thr Tyr Asn Gly Ala Leu Gly Val Asp Ile			
	385	390	395
His Glu Lys Asp His Pro Thr Ile Leu Glu Met Ala Lys Ala Ala Gly			
	405	410	415
Leu Ala Thr Gly Asn Val Ser Thr Ala Glu Leu Gln Asp Ala Thr Pro			
	420	425	430
Ala Ala Leu Val Ala His Val Thr Ser Arg Lys Cys Tyr Gly Pro Ser			
	435	440	445
Ala Thr Ser Glu Lys Cys Pro Gly Asn Ala Leu Glu Lys Gly Gly Lys			
	450	455	460
Gly Ser Ile Thr Glu Gln Leu Leu Asn Ala Arg Ala Asp Val Thr Leu			
	465	470	475
Gly Gly Gly Ala Lys Thr Phe Ala Glu Thr Ala Thr Ala Gly Glu Trp			
	485	490	495
Gln Gly Lys Thr Leu Arg Glu Gln Ala Gln Ala Arg Gly Tyr Gln Leu			
	500	505	510
Val Ser Asp Ala Ala Ser Leu Asn Ser Val Thr Glu Ala Asn Gln Gln			
	515	520	525
Lys Pro Leu Leu Gly Leu Phe Ala Asp Gly Asn Met Pro Val Arg Trp			
	530	535	540
Leu Gly Pro Lys Ala Thr Tyr His Gly Asn Ile Asp Lys Pro Ala Val			
	545	550	555
Thr Cys Thr Pro Asn Pro Gln Arg Asn Asp Ser Val Pro Thr Leu Ala			
	565	570	575
Gln Met Thr Asp Lys Ala Ile Glu Leu Leu Ser Lys Asn Glu Lys Gly			
	580	585	590
Phe Phe Leu Gln Val Glu Gly Ala Ser Ile Asp Lys Gln Asp His Ala			
	595	600	605
Ala Asn Pro Cys Gly Gln Ile Gly Glu Thr Val Asp Leu Asp Glu Ala			
	610	615	620
Val Gln Arg Ala Leu Glu Phe Ala Lys Lys Glu Gly Asn Thr Leu Val			
	625	630	635
Ile Val Thr Ala Asp His Ala His Ala Ser Gln Ile Val Ala Pro Asp			
	645	650	655

US 7,033,590 B1

79

80

-continued

Thr Lys Ala Pro Gly Leu Thr Gln Ala Leu Asn Thr Lys Asp Gly Ala  
660 665 670

Val Met Val Met Ser Tyr Gly Asn Ser Glu Glu Asp Ser Gln Glu His  
675 680 685

Thr Gly Ser Gln Leu Arg Ile Ala Ala Tyr Gly Pro His Ala Ala Asn  
690 695 700

Val Val Gly Leu Thr Asp Gln Thr Asp Leu Phe Tyr Thr Met Lys Ala  
705 710 715 720

Ala Leu Gly Asp Ile Ala His His His His His His  
725 730

<210> SEQ ID NO 91  
<211> LENGTH: 978  
<212> TYPE: DNA  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Description of Artificial Sequence:plasmid  
pZip-198AB2#102  
<220> FEATURE:  
<221> NAME/KEY: modified\_base  
<222> LOCATION: (1)..(978)  
<223> OTHER INFORMATION: n = g, a, c or t

<400> SEQUENCE: 91

atgaaatacc tattgcctac ggcagccgct ggattgttat tactcgcggc ccagccggcc 60  
atggcggagg tgaagctggg ggagctctgg ggaggcttag tgaagcctgg agggtcctcg 120  
aaactctcct gtgcagcctc tggattcact ttcagtagct ataccatgtc ttgggttcgc 180  
cagactccgg agaagaggct ggagtgggtc gcaaccatta gtagtgngg tagttccacc 240  
tactatccag acagtgtgaa gggccgattc accatctcca gagacaatgc caagaacacc 300  
ctgtacctgc aaatgagcag tctgaggtct gaggacacag ccatgtatta ctgtacaaga 360  
gaggggggtg gtttcaccgt caactgggtc ttcgatgtct ggggcgcagg aacctcagtc 420  
accgtctcct caggtggagg cggttcaggt gggcgcgccct ctggcggtgg cggatcggac 480  
attgtgctga cacagnttcc agcttctttg gctgtgtctc tagggcagag ggcaccata 540  
tcntgcagag ccagtgaag tgttgatagt tatggctata attttatgca ctggtatcag 600  
cagataccag gacagccacc caaactcctc atctatctgt catccaacct agagtctggg 660  
atccctgcca ggttcagtg cagtggtctc aggacagact tcaccctcac cattaatcct 720  
gtggaggctg atgatgttgc aacctattac tgcagcaaa gtaatgagga tccgctcacg 780  
ttcggtactg ggaccagact ggaataaaaa cgggcggccg caccgaagcc ttccactccg 840  
cccgggtctt ccggtatgaa acagctggaa gacaaagtag aggagctcct tagcaagaac 900  
taccatctag aaaacgaggt agctcgtctg aaaaagcttg ttggtgaacg tgggtgtcac 960  
catcaccatc accattaa 978

<210> SEQ ID NO 92  
<211> LENGTH: 325  
<212> TYPE: PRT  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Description of Artificial Sequence:plasmid  
pZip-198AB2#102  
<220> FEATURE:  
<221> NAME/KEY: MOD\_RES  
<222> LOCATION: (166)  
<223> OTHER INFORMATION: Xaa = Cys, Tyr, Ser or Phe

<400> SEQUENCE: 92

US 7,033,590 B1

81

82

-continued

Met Lys Tyr Leu Leu Pro Thr Ala Ala Ala Gly Leu Leu Leu Leu Ala  
 1 5 10 15  
 Ala Gln Pro Ala Met Ala Glu Val Lys Leu Val Glu Ser Gly Gly Gly  
 20 25 30  
 Leu Val Lys Pro Gly Gly Ser Leu Lys Leu Ser Cys Ala Ala Ser Gly  
 35 40 45  
 Phe Thr Phe Ser Ser Tyr Thr Met Ser Trp Val Arg Gln Thr Pro Glu  
 50 55 60  
 Lys Arg Leu Glu Trp Val Ala Thr Ile Ser Ser Gly Gly Ser Ser Thr  
 65 70 75 80  
 Tyr Tyr Pro Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn  
 85 90 95  
 Ala Lys Asn Thr Leu Tyr Leu Gln Met Ser Ser Leu Arg Ser Glu Asp  
 100 105 110  
 Thr Ala Met Tyr Tyr Cys Thr Arg Glu Gly Gly Gly Phe Thr Val Asn  
 115 120 125  
 Trp Tyr Phe Asp Val Trp Gly Ala Gly Thr Ser Val Thr Val Ser Ser  
 130 135 140  
 Gly Gly Gly Gly Ser Gly Gly Arg Ala Ser Gly Gly Gly Gly Ser Asp  
 145 150 155 160  
 Ile Val Leu Thr Gln Xaa Pro Ala Ser Leu Ala Val Ser Leu Gly Gln  
 165 170 175  
 Arg Ala Thr Ile Ser Cys Arg Ala Ser Glu Ser Val Asp Ser Tyr Gly  
 180 185 190  
 Tyr Asn Phe Met His Trp Tyr Gln Gln Ile Pro Gly Gln Pro Pro Lys  
 195 200 205  
 Leu Leu Ile Tyr Arg Ala Ser Asn Leu Glu Ser Gly Ile Pro Ala Arg  
 210 215 220  
 Phe Ser Gly Ser Gly Ser Arg Thr Asp Phe Thr Leu Thr Ile Asn Pro  
 225 230 235 240  
 Val Glu Ala Asp Asp Val Ala Thr Tyr Tyr Cys Gln Gln Ser Asn Glu  
 245 250 255  
 Asp Pro Leu Thr Phe Gly Thr Gly Thr Arg Leu Glu Ile Lys Arg Ala  
 260 265 270  
 Ala Ala Pro Lys Pro Ser Thr Pro Pro Gly Ser Ser Arg Met Lys Gln  
 275 280 285  
 Leu Glu Asp Lys Val Glu Glu Leu Leu Ser Lys Asn Tyr His Leu Glu  
 290 295 300  
 Asn Glu Val Ala Arg Leu Lys Lys Leu Val Gly Glu Arg Gly Gly His  
 305 310 315 320  
 His His His His His  
 325

<210> SEQ ID NO 93  
 <211> LENGTH: 2190  
 <212> TYPE: DNA  
 <213> ORGANISM: Artificial Sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: Description of Artificial Sequence:mAB#8860  
 scFv-alkaline phosphatase fusion protein (vector construct  
 pDAP2-8860scFv#11)

<400> SEQUENCE: 93

atgaaatacc tattgcctac ggcagccgct ggattgttat tactcgcggc ccagccggcc 60  
 atggccgagg ttcagcttca gcagtctgga cctgagctgg tgaagcccg ggccctcagtg 120

US 7,033,590 B1

83

84

-continued

```

aagatttcct gcaaagcttc tggctacgca ttcagtagct cttggatgaa ctgggtgaag 180
cagaggcctg gacagggtct tgagtggatt ggacggattt atcctggaaa tggagatact 240
aactacaatg ggaagttcaa gggcaaggcc aactgactg cagacaaatc ctccagcaca 300
gcctacatgc agctcagcag cctgacctct gtggactctg cggctctatt ctgtgcagat 360
ggtaacgtat attactatgc tatggactac tggggccaag gaacctcagt caccgtctcc 420
tcagggtggag gcggttcagg tgggcccgc tctggcgggt gcggatcgca aattgttctc 480
accaggtctc ctgcttcctt agctgtatct ctggggcaga gggccacat ctcatgcagg 540
gccagcaaaa gtgtcagtag atctggctat agttatatgc actggtacca acagaaacca 600
ggacagccac ccaaaatcct catctatctt gcatccaacc tagaatctgg ggtccctgcc 660
aggttcagtg gcagtggttc tgggacagac ttcacctca acatccatcc tgtggaggag 720
gaggatgctg caacctatta ctgtcagcac agtagggagc ttctcggac gttcggtgga 780
ggaccaaaag tggaaatcaa acgggcccgc gcagcccggg caccagaaat gcctgttctg 840
gaaaaccggg ctgctcaggg cgatattact gcacccggcg gtgctcgcg ttaaacgggt 900
gatcagactg ccgctctgct tgattctctt agcgataaac ctgcaaaaaa tattattttg 960
ctgattggcg atgggatggg ggactcggaa attactgccg cactgaatta tgccgaaggt 1020
gcgggcccgt tttttaagg tatagatgcc ttaccgctta ccgggcaata cactcactat 1080
gcgctgaata aaaaaaccgg caaacggac tacgtcaccg actcggctgc atcagcaacc 1140
gcctggtcaa ccggtgtcaa aacctataac ggcgcgctgg gcgtcgatat tcacgaaaaa 1200
gatcacccaa cgattctgga atggcaaaa gccgcaggtc tggcgaccgg taacgtttct 1260
accgcagagt tgcaggatgc cagccccgct gcgctggtgg cacatgtgac ctgcgcgaaa 1320
tgctacggtc cgagcgcgac cagtgaaaaa tgtccgggta acgctctgga aaaaggcgga 1380
aaaggatcga ttaccgaaca gctgcttaac gctcgtgccc acgttacgct tggcggcggc 1440
gcaaaaacct ttgctgaaac ggcaaccgct ggtgaatggc agggaaaaac gctgcgtgaa 1500
caggcacagg gcggtgggta tcagttgggt agcgatgctg cctcactgaa ttccggtgacg 1560
gaagcgaatc agcaaaaacc cctgcttgcc ctgtttctg acggcaatat gccagtgccg 1620
tggttaggac cgaagcaaac gtaccatggc aatatcgata agcccgagc cactgtacg 1680
ccaaatccgc aacgtaatga cagtgtacca accctggcgc agatgaccga caaagccatt 1740
gaattgttga gtaaaaatga gaaaggcttt ttcctgcaag ttgaagggtc gtcaatcgat 1800
aaacaggatc atgctcgcaa tccttggtgg caaattggcg agacggctga tctcgatgaa 1860
gccgtacaac gggcgctgga attcgctaaa aaggagggta acacgctggt catagtcacc 1920
gctgatcacg cccacgccag ccagattggt gcgccgata ccaaagctcc gggcctcacc 1980
caggcgctaa ataccaaaag tggcgagtg atgggtgatg gttacgggaa ctccgaagag 2040
gattcacaag aacataccgg cagtcagttg cgtattgcgg cgtatggccc gcatgccgcc 2100
aatgttggtg gactgaccga ccagaccgat ctcttctaca ccatgaaagc cgctctgggg 2160
gatatcgcac accatcacca tcaccattaa 2190

```

```

<210> SEQ ID NO 94
<211> LENGTH: 729
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:mAB#8860
scFv-alkaline phosphatase fusion protein (vector construct)

```

US 7,033,590 B1

85

86

-continued

---

pDAP2-8860scFv#11)

<400> SEQUENCE: 94

Met Lys Tyr Leu Leu Pro Thr Ala Ala Ala Gly Leu Leu Leu Leu Ala  
 1 5 10 15

Ala Gln Pro Ala Met Ala Glu Val Gln Leu Gln Gln Ser Gly Pro Glu  
 20 25 30

Leu Val Lys Pro Gly Ala Ser Val Lys Ile Ser Cys Lys Ala Ser Gly  
 35 40 45

Tyr Ala Phe Ser Ser Ser Trp Met Asn Trp Val Lys Gln Arg Pro Gly  
 50 55 60

Gln Gly Leu Glu Trp Ile Gly Arg Ile Tyr Pro Gly Asn Gly Asp Thr  
 65 70 75 80

Asn Tyr Asn Gly Lys Phe Lys Gly Lys Ala Thr Leu Thr Ala Asp Lys  
 85 90 95

Ser Ser Ser Thr Ala Tyr Met Gln Leu Ser Ser Leu Thr Ser Val Asp  
 100 105 110

Ser Ala Val Tyr Phe Cys Ala Asp Gly Asn Val Tyr Tyr Ala Met  
 115 120 125

Asp Tyr Trp Gly Gln Gly Thr Ser Val Thr Val Ser Ser Gly Gly Gly  
 130 135 140

Gly Ser Gly Gly Arg Ala Ser Gly Gly Gly Gly Ser Gln Ile Val Leu  
 145 150 155 160

Thr Gln Ser Pro Ala Ser Leu Ala Val Ser Leu Gly Gln Arg Ala Thr  
 165 170 175

Ile Ser Cys Arg Ala Ser Lys Ser Val Ser Thr Ser Gly Tyr Ser Tyr  
 180 185 190

Met His Trp Tyr Gln Gln Lys Pro Gly Gln Pro Pro Lys Leu Leu Ile  
 195 200 205

Tyr Leu Ala Ser Asn Leu Glu Ser Gly Val Pro Ala Arg Phe Ser Gly  
 210 215 220

Ser Gly Ser Gly Thr Asp Phe Thr Leu Asn Ile His Pro Val Glu Glu  
 225 230 235 240

Glu Asp Ala Ala Thr Tyr Tyr Cys Gln His Ser Arg Glu Leu Pro Arg  
 245 250 255

Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys Arg Ala Ala Ala Ala  
 260 265 270

Arg Ala Pro Glu Met Pro Val Leu Glu Asn Arg Ala Ala Gln Gly Asp  
 275 280 285

Ile Thr Ala Pro Gly Gly Ala Arg Arg Leu Thr Gly Asp Gln Thr Ala  
 290 295 300

Ala Leu Arg Asp Ser Leu Ser Asp Lys Pro Ala Lys Asn Ile Ile Leu  
 305 310 315 320

Leu Ile Gly Asp Gly Met Gly Asp Ser Glu Ile Thr Ala Ala Arg Asn  
 325 330 335

Tyr Ala Glu Gly Ala Gly Gly Phe Phe Lys Gly Ile Asp Ala Leu Pro  
 340 345 350

Leu Thr Gly Gln Tyr Thr His Tyr Ala Leu Asn Lys Lys Thr Gly Lys  
 355 360 365

Pro Asp Tyr Val Thr Asp Ser Ala Ala Ser Ala Thr Ala Trp Ser Thr  
 370 375 380

Gly Val Lys Thr Tyr Asn Gly Ala Leu Gly Val Asp Ile His Glu Lys  
 385 390 395 400

US 7,033,590 B1

87

88

-continued

Asp His Pro Thr Ile Leu Glu Met Ala Lys Ala Ala Gly Leu Ala Thr  
 405 410 415  
 Gly Asn Val Ser Thr Ala Glu Leu Gln Asp Ala Thr Pro Ala Ala Leu  
 420 425 430  
 Val Ala His Val Thr Ser Arg Lys Cys Tyr Gly Pro Ser Ala Thr Ser  
 435 440 445  
 Glu Lys Cys Pro Gly Asn Ala Leu Glu Lys Gly Gly Lys Gly Ser Ile  
 450 455 460  
 Thr Glu Gln Leu Leu Asn Ala Arg Ala Asp Val Thr Leu Gly Gly Gly  
 465 470 475 480  
 Ala Lys Thr Phe Ala Glu Thr Ala Thr Ala Gly Glu Trp Gln Gly Lys  
 485 490 495  
 Thr Leu Arg Glu Gln Ala Gln Ala Arg Gly Tyr Gln Leu Val Ser Asp  
 500 505 510  
 Ala Ala Ser Leu Asn Ser Val Thr Glu Ala Asn Gln Gln Lys Pro Leu  
 515 520 525  
 Leu Gly Leu Phe Ala Asp Gly Asn Met Pro Val Arg Trp Leu Gly Pro  
 530 535 540  
 Lys Ala Thr Tyr His Gly Asn Ile Asp Lys Pro Ala Val Thr Cys Thr  
 545 550 555 560  
 Pro Asn Pro Gln Arg Asn Asp Ser Val Pro Thr Leu Ala Gln Met Thr  
 565 570 575  
 Asp Lys Ala Ile Glu Leu Leu Ser Lys Asn Glu Lys Gly Phe Phe Leu  
 580 585 590  
 Gln Val Glu Gly Ala Ser Ile Asp Lys Gln Asp His Ala Ala Asn Pro  
 595 600 605  
 Cys Gly Gln Ile Gly Glu Thr Val Asp Leu Asp Glu Ala Val Gln Arg  
 610 615 620  
 Ala Leu Glu Phe Ala Lys Lys Glu Gly Asn Thr Leu Val Ile Val Thr  
 625 630 635 640  
 Ala Asp His Ala His Ala Ser Gln Ile Val Ala Pro Asp Thr Lys Ala  
 645 650 655  
 Pro Gly Leu Thr Gln Ala Leu Asn Thr Lys Asp Gly Ala Val Met Val  
 660 665 670  
 Met Ser Tyr Gly Asn Ser Glu Glu Asp Ser Gln Glu His Thr Gly Ser  
 675 680 685  
 Gln Leu Arg Ile Ala Ala Tyr Gly Pro His Ala Ala Asn Val Val Gly  
 690 695 700  
 Leu Thr Asp Gln Thr Asp Leu Phe Tyr Thr Met Lys Ala Ala Leu Gly  
 705 710 715 720  
 Asp Ile Ala His His His His His His  
 725

&lt;210&gt; SEQ ID NO 95

&lt;211&gt; LENGTH: 969

&lt;212&gt; TYPE: DNA

&lt;213&gt; ORGANISM: Artificial Sequence

&lt;220&gt; FEATURE:

 <223> OTHER INFORMATION: Description of Artificial Sequence:mAB #8860  
 scFv-leucine zipper fusion protein (miniantibody vector construct  
 p8860-Zip#1.2)

&lt;400&gt; SEQUENCE: 95

atgaaataacc tattgcctac ggcagccgct ggattgttat tactcgcggc ccagccggcc 60  
 atggcggagg ttcagcttca gcagctctgga cctgagctgg tgaagcccg ggccctcagtg 120

US 7,033,590 B1

89

90

-continued

```

aagatttcct gcaaagcttc tggctacgca ttcagtagct cttggatgaa ctgggtgaag 180
cagaggcctg gacaggggtct tgagtggatt ggacggattt atcctggaaa tggagatact 240
aactacaatg ggaagttaa gggcaaggcc acactgactg cagacaaatc ctccagcaca 300
gcctacatgc agctcagcag cctgacctct gtggactctg cggctctatt ctgtgcagat 360
ggtaacgtat attactatgc tatggactac tggggccaag gaacctcagt caccgtctcc 420
tcagggtggag gcggttcagg tgggcgcgcc tctggcggtg gcggatcgca aattgtcttc 480
accagtcctc ctgcttcctt agctgtatct ctggggcaga gggccaccat ctcatgcagg 540
gccagcaaaa gtgtcagtag atctggctat agttatatgc actggtacca acagaaacca 600
ggacagccac ccaaactcct catctatctt gcatccaacc tagaatctgg ggtccctgcc 660
aggttcagtg gcagtgggtc tgggacagac ttcacctca acatccatcc tgtggaggag 720
gaggtgctg caacctatta ctgtcagcac agtagggagc ttctcggac gtccggtgga 780
ggcaccaagc tggaaatcaa acgggcggcc gcaccgaagc cttccactcc gcccggtct 840
tcccgatatg aacagctgga agacaaagta gaggagctcc ttagcaagaa ctaccatcta 900
gaaaacgagg tagctcgtct gaaaaagctt gttggtgaac gtggtggtca ccatcaccat 960
caccattaa 969

```

```

<210> SEQ ID NO 96
<211> LENGTH: 322
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:mAB #8860
scFv-leucine zipper fusion protein (miniantibody vector construct
p8860-Zip#1.2)

```

&lt;400&gt; SEQUENCE: 96

```

Met Lys Tyr Leu Leu Pro Thr Ala Ala Gly Leu Leu Leu Ala
 1             5             10             15
Ala Gln Pro Ala Met Ala Glu Val Gln Leu Gln Gln Ser Gly Pro Glu
                20             25             30
Leu Val Lys Pro Gly Ala Ser Val Lys Ile Ser Cys Lys Ala Ser Gly
                35             40             45
Tyr Ala Phe Ser Ser Ser Trp Met Asn Trp Val Lys Gln Arg Pro Gly
                50             55             60
Gln Gly Leu Glu Trp Ile Gly Arg Ile Tyr Pro Gly Asn Gly Asp Thr
        65             70             75             80
Asn Tyr Asn Gly Lys Phe Lys Gly Lys Ala Thr Leu Thr Ala Asp Lys
                85             90             95
Ser Ser Ser Thr Ala Tyr Met Gln Leu Ser Ser Leu Thr Ser Val Asp
                100            105            110
Ser Ala Val Tyr Phe Cys Ala Asp Gly Asn Val Tyr Tyr Tyr Ala Met
                115            120            125
Asp Tyr Trp Gly Gln Gly Thr Ser Val Thr Val Ser Ser Gly Gly Gly
        130            135            140
Gly Ser Gly Gly Arg Ala Ser Gly Gly Gly Ser Gln Ile Val Leu
        145            150            155            160
Thr Gln Ser Pro Ala Ser Leu Ala Val Ser Leu Gly Gln Arg Ala Thr
                165            170            175
Ile Ser Cys Arg Ala Ser Lys Ser Val Ser Thr Ser Gly Tyr Ser Tyr
                180            185            190
Met His Trp Tyr Gln Gln Lys Pro Gly Gln Pro Pro Lys Leu Leu Ile

```



US 7,033,590 B1

91

92

-continued

195	200	205	
Tyr Leu Ala Ser Asn Leu Glu Ser Gly Val Pro Ala Arg Phe Ser Gly			
210	215	220	
Ser Gly Ser Gly Thr Asp Phe Thr Leu Asn Ile His Pro Val Glu Glu			
225	230	235	240
Glu Asp Ala Ala Thr Tyr Tyr Cys Gln His Ser Arg Glu Leu Pro Arg			
	245	250	255
Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys Arg Ala Ala Ala Pro			
	260	265	270
Lys Pro Ser Thr Pro Pro Gly Ser Ser Arg Met Lys Gln Leu Glu Asp			
	275	280	285
Lys Val Glu Glu Leu Leu Ser Lys Asn Tyr His Leu Glu Asn Glu Val			
	290	295	300
Ala Arg Leu Lys Lys Leu Val Gly Glu Arg Gly Gly His His His His			
305	310	315	320
His His			
<210> SEQ ID NO 97			
<211> LENGTH: 270			
<212> TYPE: DNA			
<213> ORGANISM: Artificial Sequence			
<220> FEATURE:			
<223> OTHER INFORMATION: Description of Artificial Sequence:part of plasmid pMycHis6 differing from vector pCOCK			
<400> SEQUENCE: 97			
caggaacag ctatgacct gattacgcc agcttccatg aaaattctat ttcaaggaga			60
cagtcataat gaaataccta ttgcctacgg cagccgctgg attgttatta ctgcgggccc			120
agccggccat ggcccagggtg cagctgcagg cgcgcctgca ggtcgacctc gagatcaaac			180
ggcgcgccgc agaacaaaaa ctcatctcag aagaggatct gaatggggcg gcacatcacc			240
atcaccatca ctaataagaa ttacttgcc			270
<210> SEQ ID NO 98			
<211> LENGTH: 61			
<212> TYPE: PRT			
<213> ORGANISM: Artificial Sequence			
<220> FEATURE:			
<223> OTHER INFORMATION: Description of Artificial Sequence:part of plasmid pMycHis6 differing from vector pCOCK			
<400> SEQUENCE: 98			
Met Lys Tyr Leu Leu Pro Thr Ala Ala Ala Gly Leu Leu Leu Ala			
1	5	10	15
Ala Gln Pro Ala Met Ala Gln Val Gln Leu Gln Ala Arg Leu Gln Val			
	20	25	30
Asp Leu Glu Ile Lys Arg Ala Ala Ala Glu Gln Lys Leu Ile Ser Glu			
	35	40	45
Glu Asp Leu Asn Gly Ala Ala His His His His His His			
	50	55	60
<210> SEQ ID NO 99			
<211> LENGTH: 888			
<212> TYPE: DNA			
<213> ORGANISM: Artificial Sequence			
<220> FEATURE:			
<223> OTHER INFORMATION: Description of Artificial Sequence:198AB2 scFv linked to c-myc-tag and His6 tag (ORF of expression vector pMycHis6-198AB2#102)			
<220> FEATURE:			

US 7,033,590 B1

93

94

-continued

&lt;221&gt; NAME/KEY: modified\_base

&lt;222&gt; LOCATION: (228)

&lt;223&gt; OTHER INFORMATION: n = g, a, c or t

&lt;400&gt; SEQUENCE: 99

```

atgaaatacc tattgcctac ggcagccgct ggattgttat tactcggggc ccagccggcc    60
atggccgagg tgaagctggt ggagtctggg ggaggcttag tgaagcctgg agggccctg    120
aaactctcct gtgcagcctc tggattcact ttcagtagct ataccatgtc ttgggttcgc    180
cagactccgg agaagaggct ggagtgggtc gcaaccatta gtagtgnggg tagttccacc    240
tactatccag acagtgtgaa gggccgattc accatctcca gagacaatgc caagaacacc    300
ctgtacctgc aaatgagcag tctgaggtct gaggacacag ccatgtatta ctgtacaaga    360
gagggggggt gtttcaccgt caactgggtac ttcgatgtct ggggcgcagg aacctcagtc    420
accgtctcct caggtggagg cggttcaggt gggcgcgcct ctggcgggtg cggtatcgac    480
attgtgtga cacagtctcc agcttctttg gctgtgtctc tagggcagag ggcaccata    540
tctcgagag ccagtgaag tgttgatagt tatggctata attttatgca ctggtatcag    600
cagataccag gacagccacc caaactcctc atctatcgtg catccaacct agagtctggg    660
atccctgcc ggttcagtg gctgggtct aggacagact tcaccctcac cattaatcct    720
gtggaggctg atgatgttg aacctattac tgtcagcaaa gtaatgagga tccgctcacg    780
ttcggtactg ggaccagact gaaataaaaa cgggcgggccc cagaacaaaa actcatctca    840
gaagaggatc tgaatggggc ggcacatcac catcaccatc actaataa    888

```

&lt;210&gt; SEQ ID NO 100

&lt;211&gt; LENGTH: 294

&lt;212&gt; TYPE: PRT

&lt;213&gt; ORGANISM: Artificial Sequence

&lt;220&gt; FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence:198AB2 scFv  
linked to c-myc-tag and His6 tag (ORF of expression vector  
pMycHis6-198AB2#102)

&lt;400&gt; SEQUENCE: 100

```

Met Lys Tyr Leu Leu Pro Thr Ala Ala Ala Gly Leu Leu Leu Ala
 1             5             10             15
Ala Gln Pro Ala Met Ala Glu Val Lys Leu Val Glu Ser Gly Gly Gly
 20             25             30
Leu Val Lys Pro Gly Gly Ser Leu Lys Leu Ser Cys Ala Ala Ser Gly
 35             40             45
Phe Thr Phe Ser Ser Tyr Thr Met Ser Trp Val Arg Gln Thr Pro Glu
 50             55             60
Lys Arg Leu Glu Trp Val Ala Thr Ile Ser Ser Gly Gly Ser Ser Thr
 65             70             75             80
Tyr Tyr Pro Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn
 85             90             95
Ala Lys Asn Thr Leu Tyr Leu Gln Met Ser Ser Leu Arg Ser Glu Asp
100            105            110
Thr Ala Met Tyr Tyr Cys Thr Arg Glu Gly Gly Gly Phe Thr Val Asn
115            120            125
Trp Tyr Phe Asp Val Trp Gly Ala Gly Thr Ser Val Thr Val Ser Ser
130            135            140
Gly Gly Gly Gly Ser Gly Gly Arg Ala Ser Gly Gly Gly Gly Ser Asp
145            150            155            160
Ile Val Leu Thr Gln Ser Pro Ala Ser Leu Ala Val Ser Leu Gly Gln

```

US 7,033,590 B1

95

96

-continued

165										170										175										
Arg	Ala	Thr	Ile	Ser	Cys	Arg	Ala	Ser	Glu	Ser	Val	Asp	Ser	Tyr	Gly															
			180						185					190																
Tyr	Asn	Phe	Met	His	Trp	Tyr	Gln	Gln	Ile	Pro	Gly	Gln	Pro	Pro	Lys															
	195						200					205																		
Leu	Leu	Ile	Tyr	Arg	Ala	Ser	Asn	Leu	Glu	Ser	Gly	Ile	Pro	Ala	Arg															
	210					215					220																			
Phe	Ser	Gly	Ser	Gly	Ser	Arg	Thr	Asp	Phe	Thr	Leu	Thr	Ile	Asn	Pro															
225					230					235					240															
Val	Glu	Ala	Asp	Asp	Val	Ala	Thr	Tyr	Tyr	Cys	Gln	Gln	Ser	Asn	Glu															
			245						250					255																
Asp	Pro	Leu	Thr	Phe	Gly	Thr	Gly	Thr	Arg	Leu	Glu	Ile	Lys	Arg	Ala															
		260					265						270																	
Ala	Ala	Glu	Gln	Lys	Leu	Ile	Ser	Glu	Glu	Asp	Leu	Asn	Gly	Ala	Ala															
		275					280					285																		
His	His	His	His	His	His																									
	290																													

<210> SEQ ID NO 101  
 <211> LENGTH: 876  
 <212> TYPE: DNA  
 <213> ORGANISM: Artificial Sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: Description of Artificial Sequence:mAB #8860  
 scFv linked to c-myc-tag and His6-tag designated 8860-M/H#4c  
 (plasmid vector p8860-M/H#4c)

<400> SEQUENCE: 101

atgaaataacc	tattgcctac	ggcagccgct	ggattgttat	tactcgcggc	ccagccggcc	60
atggccgagg	ttcagcttca	gcagtctgga	cctgagctgg	tgaagcccg	ggcctcagtg	120
aagatttcct	gcaaagcttc	tggtctacga	ttcagtagct	cttgatgaa	ctgggtgaag	180
cagagccctg	gacagggctc	tgagtggatt	ggacggattt	atcctggaaa	tgagataact	240
aactacaatg	ggaagttaa	gggcaaggcc	acactgactg	cagacaaatc	ctccagcaca	300
gcctacatgc	agctcagcag	cctgacctct	gtggactctg	cggtctattt	ctgtgcagat	360
ggtaacgtat	attactatgc	tatggactac	tggggccaag	gaacctcagt	caccgtctcc	420
tcaggtggag	gcggttcagg	tgggcgcgcc	tctggcggtg	gcggatcgca	aattgttctc	480
acccagtctc	ctgcttcctt	agctgtatct	ctggggcaga	gggccaccat	ctcatgcagg	540
gccagcaaaa	gtgtcagtac	atctggctat	agttatatgc	actggtacca	acagaaacca	600
ggacagccac	ccaaactcct	catctatctt	gcattcaacc	tagaatctgg	ggtccctgcc	660
aggttcagtg	gcagtgggtc	tgggacagac	ttcacctcca	acatccatcc	tgtggaggag	720
gaggatgctg	caacctatta	ctgtcagcac	agtagggagc	ttcctcggac	gttcggtgga	780
ggcacaagc	tggaaatcaa	acgggcggcc	gcagaacaaa	aactcatctc	agaagaggat	840
ctgaatgggg	cggcacatca	ccatcaccat	cactaa			876

<210> SEQ ID NO 102  
 <211> LENGTH: 291  
 <212> TYPE: PRT  
 <213> ORGANISM: Artificial Sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: Description of Artificial Sequence:mAB #8860  
 scFv linked to c-myc-tag and His6-tag designated 8860-M/H#4c  
 (plasmid vector p8860-M/H#4c)

<400> SEQUENCE: 102

US 7,033,590 B1

97

98

-continued

Met Lys Tyr Leu Leu Pro Thr Ala Ala Ala Gly Leu Leu Leu Leu Ala  
1 5 10 15  
Ala Gln Pro Ala Met Ala Glu Val Gln Leu Gln Gln Ser Gly Pro Glu  
20 25 30  
Leu Val Lys Pro Gly Ala Ser Val Lys Ile Ser Cys Lys Ala Ser Gly  
35 40 45  
Tyr Ala Phe Ser Ser Ser Trp Met Asn Trp Val Lys Gln Arg Pro Gly  
50 55 60  
Gln Gly Leu Glu Trp Ile Gly Arg Ile Tyr Pro Gly Asn Gly Asp Thr  
65 70 75 80  
Asn Tyr Asn Gly Lys Phe Lys Gly Lys Ala Thr Leu Thr Ala Asp Lys  
85 90 95  
Ser Ser Ser Thr Ala Tyr Met Gln Leu Ser Ser Leu Thr Ser Val Asp  
100 105 110  
Ser Ala Val Tyr Phe Cys Ala Asp Gly Asn Val Tyr Tyr Tyr Ala Met  
115 120 125  
Asp Tyr Trp Gly Gln Gly Thr Ser Val Thr Val Ser Ser Gly Gly Gly  
130 135 140  
Gly Ser Gly Gly Arg Ala Ser Gly Gly Gly Gly Ser Gln Ile Val Leu  
145 150 155 160  
Thr Gln Ser Pro Ala Ser Leu Ala Val Ser Leu Gly Gln Arg Ala Thr  
165 170 175  
Ile Ser Cys Arg Ala Ser Lys Ser Val Ser Thr Ser Gly Tyr Ser Tyr  
180 185 190  
Met His Trp Tyr Gln Gln Lys Pro Gly Gln Pro Pro Lys Leu Leu Ile  
195 200 205  
Tyr Leu Ala Ser Asn Leu Glu Ser Gly Val Pro Ala Arg Phe Ser Gly  
210 215 220  
Ser Gly Ser Gly Thr Asp Phe Thr Leu Asn Ile His Pro Val Glu Glu  
225 230 235 240  
Glu Asp Ala Ala Thr Tyr Tyr Cys Gln His Ser Arg Glu Leu Pro Arg  
245 250 255  
Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys Arg Ala Ala Glu  
260 265 270  
Gln Lys Leu Ile Ser Glu Glu Asp Leu Asn Gly Ala Ala His His His  
275 280 285  
His His His  
290

<210> SEQ ID NO 103  
<211> LENGTH: 74  
<212> TYPE: DNA  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Description of Artificial Sequence:annealed  
oligonucleotide

<400> SEQUENCE: 103

ggcgcgagaa caaaaactca tctcagaaga ggatctgaat ggggcggcac atcaccatca 60  
ccatcactaa taag 74

<210> SEQ ID NO 104  
<211> LENGTH: 69  
<212> TYPE: DNA  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:

US 7,033,590 B1

99

100

-continued

---

<223> OTHER INFORMATION: Description of Artificial Sequence:annealed oligonucleotide

<400> SEQUENCE: 104

ttattagtga tggatgatgg gatgtgccgc ccattcaga tcctcttctg agatgagttt 60

ttgttctgc 69

<210> SEQ ID NO 105

<211> LENGTH: 16

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence:CDR3 peptide

<220> FEATURE:

<221> NAME/KEY: MOD\_RES

<222> LOCATION: (1)..(16)

<223> OTHER INFORMATION: Xaa = any amino acid

<400> SEQUENCE: 105

Cys Xaa Xaa Tyr Gly Asn Ser Pro Lys Gly Phe Ala Tyr Xaa Xaa Cys

1 5 10 15

<210> SEQ ID NO 106

<211> LENGTH: 16

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence:CDR3 peptide

<400> SEQUENCE: 106

Phe Arg Asn Arg Gly Met Thr Ala Leu Leu Lys Val Ser Ser Cys Asp

1 5 10 15

<210> SEQ ID NO 107

<211> LENGTH: 30

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence:portion of plasmid pMycHis6 with pelB-leader, polylinker and c-myc tag

<400> SEQUENCE: 107

Leu Ala Ala Gln Pro Ala Met Ala Gln Val Gln Leu Gln Ala Arg Leu

1 5 10 15

Gln Val Asp Leu Glu Ile Lys Arg Ala Ala Ala Glu Gln Lys

20 25 30

<210> SEQ ID NO 108

<211> LENGTH: 90

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence:portion of plasmid pMycHis6 with pelB-leader, polylinker and c-myc tag

<400> SEQUENCE: 108

ctcgcggccc agccggccat ggcccagggt cagctgcagg cgcgcctgca ggtcgacctc 60

gagatcaaac gggcgggccg agaacaaaaa 90

<210> SEQ ID NO 109

<211> LENGTH: 12

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence:c-myc-tag

US 7,033,590 B1

101

102

-continued

&lt;400&gt; SEQUENCE: 109

Glu Gln Lys Leu Ile Ser Glu Glu Asp Leu Asn Gly  
 1 5 10

&lt;210&gt; SEQ ID NO 110

&lt;211&gt; LENGTH: 6

&lt;212&gt; TYPE: PRT

&lt;213&gt; ORGANISM: Artificial Sequence

&lt;220&gt; FEATURE:

&lt;223&gt; OTHER INFORMATION: Description of Artificial Sequence:His6-tag

&lt;400&gt; SEQUENCE: 110

His His His His His His  
 1 5

&lt;210&gt; SEQ ID NO 111

&lt;211&gt; LENGTH: 15

&lt;212&gt; TYPE: PRT

&lt;213&gt; ORGANISM: Artificial Sequence

&lt;220&gt; FEATURE:

&lt;223&gt; OTHER INFORMATION: Description of Artificial Sequence:flexible linker

&lt;400&gt; SEQUENCE: 111

Gly Gly Gly Gly Ser Gly Gly Arg Ala Ser Gly Gly Gly Gly Ser  
 1 5 10 15

&lt;210&gt; SEQ ID NO 112

&lt;211&gt; LENGTH: 12

&lt;212&gt; TYPE: PRT

&lt;213&gt; ORGANISM: Artificial Sequence

&lt;220&gt; FEATURE:

&lt;223&gt; OTHER INFORMATION: Description of Artificial Sequence:Al peptide core sequence

&lt;400&gt; SEQUENCE: 112

Glu Gly Gly Gly Tyr Tyr Val Asn Trp Tyr Phe Asp  
 1 5 10

What is claimed is:

1. An isolated antibody or antibody fragment thereof that binds Factor IX or Factor IXa and increases the procoagulant activity of Factor IXa.

2. The antibody or antibody fragment according to claim 1 that increases the procoagulant activity of Factor IXa in the presence of Factor VIII inhibitors.

3. The antibody or antibody fragment according to claim 1 wherein the antibody is an IgG, IgM, IgA or IgE antibody.

4. The antibody or antibody fragment according to claim 1, wherein said antibody or antibody fragment is selected from the group consisting of a monoclonal antibody, a chimeric antibody, a humanized antibody, a single chain antibody, a bispecific antibody, a diabody, and di-, oligo- or multimers thereof.

5. A CDR3 peptide of the antibody or antibody fragment according to claim 1 consisting of an amino acid sequence selected from the group consisting of:

Tyr-Gly-Asn-Ser-Pro-Lys-Gly-Phe-Ala-Tyr (SEQ ID NO:5); and

Asp-Gly-Gly-His-Gly-Tyr-Gly-Ser-Ser-Phe-Asp-Tyr (SEQ ID NO:6).

6. The antibody or antibody fragment according to claim 1, wherein the variable region of said antibody or antibody fragment comprises amino acids 1-119 and amino acids 135-242 as listed in SEQ ID NO:82.

7. The antibody or antibody fragment according to claim 6 that additionally comprises an artificial linker sequence.

8. The antibody or antibody fragment according to claim 1, wherein the variable region of said antibody or antibody fragment comprises amino acids 1-121 and amino acids 137-249 as listed in SEQ ID NO:84.

9. The antibody or antibody fragment according to claim 8 that additionally comprises an artificial linker sequence.

10. The antibody or antibody fragment according to claim 1, wherein the variable region of said antibody or antibody fragment comprises amino acids 1-122 and amino acids 138-249 as listed in SEQ ID NO:86.

11. The antibody or antibody fragment according to claim 10 that additionally comprises an artificial linker sequence.

12. A hybridoma cell line secreting an antibody that binds Factor IX or Factor IXa and increases the procoagulant activity of Factor IXa.

13. The hybridoma cell line according to claim 12 that is selected from the group consisting of cell lines having ECACC deposit numbers 99090924, 99090925, 99090926, 99121614, 99121615, 99121616, 99121617, 99121618, 99121619 and 99121620.

14. An antibody that is secreted by a hybridoma cell line according to claim 12.

15. A preparation comprising an antibody or antibody fragment according to claim 1 and a pharmaceutically acceptable carrier.

US 7,033,590 B1

103

16. The preparation according to claim 15, additionally comprising Factor IXa $\alpha$  and/or Factor IXa $\beta$ .

17. A method of obtaining an antibody that interacts with Factor IX or Factor IXa and increases the procoagulant activity of Factor IXa, comprising the steps of:

immunizing an immunocompetent mouse with an antigen selected from the group consisting of FIX, FIXa $\alpha$ , FIXa $\beta$  or fragments thereof,

isolating spleen cells of the immunized mouse,

producing hybridoma cells,

screening the hybridoma cell supernatants for an increase in the procoagulant activity of Factor IXa, isolating and purifying the antibody from a supernatant from the

104

hybridoma cells which exhibit an increase in the procoagulant activity of Factor IXa.

18. The antibody or antibody fragment according to claim 4, wherein the antibody fragment is a single chain antibody.

19. The antibody or antibody fragment according to claim 4, wherein the antibody is a humanized antibody.

20. The antibody or antibody fragment according to claim 2 wherein the antibody is selected from the group consisting of an IgG, IgM, IgA or IgE antibody.

21. The antibody or antibody fragment of claim 1, wherein the antibody fragment comprises a CDR3 peptide.

22. The antibody or antibody fragment of claim 1, wherein the antibody fragment is a CDR3 peptide.

\* \* \* \* \*

APPEAL,PATENT

**U.S. District Court  
District of Delaware (Wilmington)  
CIVIL DOCKET FOR CASE #: 1:17-cv-00509-TBD**

Baxalta Incorporated et al v. Genentech, Inc. et al  
Assigned to: Judge Timothy Belcher Dyk  
Case in other court: Federal Circuit, 19-01527  
Federal Circuit, 22-01461

Date Filed: 05/04/2017  
Jury Demand: Plaintiff  
Nature of Suit: 830 Patent  
Jurisdiction: Federal Question

Cause: 35:271 Patent Infringement

**Plaintiff**

**Baxalta Incorporated**  
*a Delaware corporation*

represented by **Jody Barillare**  
Philadelphia  
1201 N. Market Street  
Ste 2201  
Wilmington, DE 19801  
302-574-7294  
Email: [jody.barillare@morganlewis.com](mailto:jody.barillare@morganlewis.com)  
**LEAD ATTORNEY**  
**ATTORNEY TO BE NOTICED**

**Michael J. Abernathy**  
Email: [mike.abernathy@morganlewis.com](mailto:mike.abernathy@morganlewis.com)  
**LEAD ATTORNEY**  
**PRO HAC VICE**  
**ATTORNEY TO BE NOTICED**

**Amanda S. Williamson**  
Email: [amanda.williamson@morganlewis.com](mailto:amanda.williamson@morganlewis.com)  
**PRO HAC VICE**  
**ATTORNEY TO BE NOTICED**

**Amy Michele Dudash**  
Morgan Lewis & Bockius LLP  
1007 Orange Street  
Suite 501  
Wilmington, DE 19801  
302-574-7293  
Email: [amy.dudash@morganlewis.com](mailto:amy.dudash@morganlewis.com)  
**ATTORNEY TO BE NOTICED**

**Caroline Lourgous**  
Email: [caroline.lourgous@kirkland.com](mailto:caroline.lourgous@kirkland.com)  
**PRO HAC VICE**  
**ATTORNEY TO BE NOTICED**

**Colm F. Connolly**  
Morgan Lewis & Bockius LLP  
1007 Orange Street  
Suite 501  
Wilmington, DE 19801  
(302) 574-3000  
Fax: (302) 574-3001  
Email: [colm.connolly@morganlewis.com](mailto:colm.connolly@morganlewis.com)  
**TERMINATED: 11/07/2018**

**Jason C. White**  
Email: [jason.white@morganlewis.com](mailto:jason.white@morganlewis.com)  
**PRO HAC VICE**  
**ATTORNEY TO BE NOTICED**

**Jennifer M. Dienes**



UNDELIVERABLE EMAIL 2/12/20  
*PRO HAC VICE*  
*ATTORNEY TO BE NOTICED*

**Jesse T. Dyer**  
UNDELIVERABLE EMAIL 2/22/2021  
*PRO HAC VICE*  
*ATTORNEY TO BE NOTICED*

**Karon N. Fowler**  
Email: [karon.fowler@morganlewis.com](mailto:karon.fowler@morganlewis.com)  
*PRO HAC VICE*  
*ATTORNEY TO BE NOTICED*

**Maria E. Doukas**  
Email: [maria.doukas@morganlewis.com](mailto:maria.doukas@morganlewis.com)  
*PRO HAC VICE*  
*ATTORNEY TO BE NOTICED*

**Olga Berson**  
Email: [olga.berson@morganlewis.com](mailto:olga.berson@morganlewis.com)  
*PRO HAC VICE*  
*ATTORNEY TO BE NOTICED*

**Wan-Shon Lo**  
Email: [shon.lo@morganlewis.com](mailto:shon.lo@morganlewis.com)  
*PRO HAC VICE*  
*ATTORNEY TO BE NOTICED*

**William R. Peterson**  
Email: [william.peterson@morganlewis.com](mailto:william.peterson@morganlewis.com)  
*PRO HAC VICE*  
*ATTORNEY TO BE NOTICED*

**Plaintiff**

**Baxalta GmbH**  
*a Swiss company*

represented by **Jody Barillare**  
(See above for address)  
*LEAD ATTORNEY*  
*ATTORNEY TO BE NOTICED*

**Amanda S. Williamson**  
(See above for address)  
*PRO HAC VICE*  
*ATTORNEY TO BE NOTICED*

**Amy Michele Dudash**  
(See above for address)  
*ATTORNEY TO BE NOTICED*

**Caroline Lourgou**  
(See above for address)  
*PRO HAC VICE*  
*ATTORNEY TO BE NOTICED*

**Colm F. Connolly**  
(See above for address)  
*TERMINATED: 11/07/2018*

**Jason C. White**  
(See above for address)  
*PRO HAC VICE*  
*ATTORNEY TO BE NOTICED*

**Jennifer M. Dienes**

(See above for address)  
*PRO HAC VICE*  
*ATTORNEY TO BE NOTICED*

**Jesse T. Dyer**  
(See above for address)  
*PRO HAC VICE*  
*ATTORNEY TO BE NOTICED*

**Karon N. Fowler**  
(See above for address)  
*PRO HAC VICE*  
*ATTORNEY TO BE NOTICED*

**Maria E. Doukas**  
(See above for address)  
*PRO HAC VICE*  
*ATTORNEY TO BE NOTICED*

**Olga Berson**  
(See above for address)  
*PRO HAC VICE*  
*ATTORNEY TO BE NOTICED*

**Sanjay K. Murthy**  
UNDELIVERABLE EMAIL 2/9/2022  
*PRO HAC VICE*  
*ATTORNEY TO BE NOTICED*

**Wan-Shon Lo**  
(See above for address)  
*PRO HAC VICE*  
*ATTORNEY TO BE NOTICED*

**William R. Peterson**  
(See above for address)  
*PRO HAC VICE*  
*ATTORNEY TO BE NOTICED*

V.

**Defendant**

**Genentech, Inc.**  
*a Delaware corporation*

represented by **Steven J. Balick**  
Ashby & Geddes  
500 Delaware Avenue, 8th Floor  
P.O. Box 1150  
Wilmington, DE 19801  
(302) 654-1888  
Fax: (302) 654-2067  
Email: [sbalick@ashbygeddes.com](mailto:sbalick@ashbygeddes.com)  
*LEAD ATTORNEY*  
*ATTORNEY TO BE NOTICED*

**Alexander F. Atkins**  
Email: [aatkins@paulweiss.com](mailto:aatkins@paulweiss.com)  
*TERMINATED: 01/12/2022*  
*PRO HAC VICE*

**Andrew Colin Mayo**  
Ashby & Geddes  
500 Delaware Avenue, 8th Floor  
P.O. Box 1150  
Wilmington, DE 19801  
302-654-1888

Email: [amayo@ashbygeddes.com](mailto:amayo@ashbygeddes.com)  
*ATTORNEY TO BE NOTICED*

**Catherine Nyarady**  
Email: [cnyarady@paulweiss.com](mailto:cnyarady@paulweiss.com)  
*PRO HAC VICE*  
*ATTORNEY TO BE NOTICED*

**David E. Cole**  
Email: [dcole@paulweiss.com](mailto:dcole@paulweiss.com)  
*PRO HAC VICE*  
*ATTORNEY TO BE NOTICED*

**Eric A. Stone**  
Email: [estone@paulweiss.com](mailto:estone@paulweiss.com)  
*PRO HAC VICE*  
*ATTORNEY TO BE NOTICED*

**Jennifer Gordon**  
Email: [jengordon@paulweiss.com](mailto:jengordon@paulweiss.com)  
*PRO HAC VICE*  
*ATTORNEY TO BE NOTICED*

**Josephine Young**  
Email: [jyoung@paulweiss.com](mailto:jyoung@paulweiss.com)  
*PRO HAC VICE*  
*ATTORNEY TO BE NOTICED*

**Kenneth A. Gallo**  
Email: [kgallo@paulweiss.com](mailto:kgallo@paulweiss.com)  
*PRO HAC VICE*  
*ATTORNEY TO BE NOTICED*

**Marissa C.M. Doran**  
Email: [mdoran@paulweiss.com](mailto:mdoran@paulweiss.com)  
*PRO HAC VICE*  
*ATTORNEY TO BE NOTICED*

**Naz Erdeniz Wehrli**  
Email: [nwehrli@paulweiss.com](mailto:nwehrli@paulweiss.com)  
*PRO HAC VICE*  
*ATTORNEY TO BE NOTICED*

**Nicolas P. Groombridge**  
Email: [ngroombridge@paulweiss.com](mailto:ngroombridge@paulweiss.com)  
*PRO HAC VICE*  
*ATTORNEY TO BE NOTICED*

**Defendant**

**Chugai Pharmaceutical Co., Ltd.**  
*a Japanese company*  
*TERMINATED: 09/19/2018*

represented by **John W. Shaw**  
Shaw Keller LLP  
I.M. Pei Building  
1105 North Market Street  
12th Floor  
Wilmington, DE 19801  
302-298-0701  
Email: [jshaw@shawkeller.com](mailto:jshaw@shawkeller.com)  
*LEAD ATTORNEY*

**Brian M. Kramer**  
Email: [bmramer@mofo.com](mailto:bmramer@mofo.com)  
*PRO HAC VICE*  
*ATTORNEY TO BE NOTICED*

**David C. Doyle**  
UNDELIVERABLE EMAIL 7/1/2021  
*PRO HAC VICE*  
*ATTORNEY TO BE NOTICED*

**David M. Fry**  
JPMorgan Chase & Co.  
4041 Ogletown Stanton Road  
Newark, DE 19713  
302-455-3374  
Email: [dfry@shawkeller.com](mailto:dfry@shawkeller.com)  
*ATTORNEY TO BE NOTICED*

**Karen Elizabeth Keller**  
Shaw Keller LLP  
1105 North Market Street, 12th Floor  
Wilmington, DE 19801  
302-298-0700  
Email: [kkeller@shawkeller.com](mailto:kkeller@shawkeller.com)

**Stephen D. Keane**  
UNDELIVERABLE EMAIL 10/15/2020  
*PRO HAC VICE*  
*ATTORNEY TO BE NOTICED*

**Amicus**

**Patients for Access to Advanced  
Hemophilia Therapy**

represented by **Patients for Access to Advanced  
Hemophilia Therapy**  
c/o Mark Antell  
1573-301 N. Colonial Terrace  
Arlington, VA 22209  
PRO SE

**Counter Claimant**

**Genentech, Inc.**  
*a Delaware corporation*

represented by **Steven J. Balick**  
(See above for address)  
*LEAD ATTORNEY*  
*ATTORNEY TO BE NOTICED*

V.

**Counter Defendant**

**Baxalta GmbH**  
*a Swiss company*

represented by **Amy Michele Dudash**  
(See above for address)  
*LEAD ATTORNEY*  
*ATTORNEY TO BE NOTICED*

**Amanda S. Williamson**  
(See above for address)  
*ATTORNEY TO BE NOTICED*

**Colm F. Connolly**  
(See above for address)  
*TERMINATED: 11/07/2018*

**Jennifer M. Dienes**  
(See above for address)  
*PRO HAC VICE*  
*ATTORNEY TO BE NOTICED*

**Maria E. Doukas**  
(See above for address)

ATTORNEY TO BE NOTICED

**Counter Defendant**

**Baxalta Incorporated**  
*a Delaware corporation*

represented by **Amy Michele Dudash**  
(See above for address)  
**LEAD ATTORNEY**  
**ATTORNEY TO BE NOTICED**

**Amanda S. Williamson**  
(See above for address)  
**ATTORNEY TO BE NOTICED**

**Christopher J Betti**  
Email: [christopher.betti@morganlewis.com](mailto:christopher.betti@morganlewis.com)  
**PRO HAC VICE**  
**ATTORNEY TO BE NOTICED**

**Colm F. Connolly**  
(See above for address)  
**TERMINATED: 11/07/2018**

**Jennifer M. Dienes**  
(See above for address)  
**PRO HAC VICE**  
**ATTORNEY TO BE NOTICED**

**Maria E. Doukas**  
(See above for address)  
**ATTORNEY TO BE NOTICED**

**Counter Claimant**

**Genentech, Inc.**  
*a Delaware corporation*

represented by **Steven J. Balick**  
(See above for address)  
**LEAD ATTORNEY**  
**ATTORNEY TO BE NOTICED**

**Andrew Colin Mayo**  
(See above for address)  
**ATTORNEY TO BE NOTICED**

**David E. Cole**  
(See above for address)  
**ATTORNEY TO BE NOTICED**

**Eric A. Stone**  
(See above for address)  
**ATTORNEY TO BE NOTICED**

**Jennifer Gordon**  
(See above for address)  
**PRO HAC VICE**  
**ATTORNEY TO BE NOTICED**

**Kenneth A. Gallo**  
(See above for address)  
**ATTORNEY TO BE NOTICED**

**Nicolas P. Groombridge**  
(See above for address)  
**ATTORNEY TO BE NOTICED**

**Counter Defendant**

**Baxalta GmbH**  
*a Swiss company*

represented by **Jody Barillare**  
(See above for address)  
**LEAD ATTORNEY**  
**ATTORNEY TO BE NOTICED**

**Amanda S. Williamson**  
(See above for address)  
**ATTORNEY TO BE NOTICED**

**Amy Michele Dudash**  
(See above for address)  
**ATTORNEY TO BE NOTICED**

**Caroline Lourgous**  
(See above for address)  
**PRO HAC VICE**  
**ATTORNEY TO BE NOTICED**

**Colm F. Connolly**  
(See above for address)  
**TERMINATED: 11/07/2018**

**Jason C. White**  
(See above for address)  
**ATTORNEY TO BE NOTICED**

**Jennifer M. Dienes**  
(See above for address)  
**PRO HAC VICE**  
**ATTORNEY TO BE NOTICED**

**Jesse T. Dyer**  
(See above for address)  
**PRO HAC VICE**  
**ATTORNEY TO BE NOTICED**

**Karon N. Fowler**  
(See above for address)  
**ATTORNEY TO BE NOTICED**

**Maria E. Doukas**  
(See above for address)  
**ATTORNEY TO BE NOTICED**

**Sanjay K. Murthy**  
(See above for address)  
**PRO HAC VICE**  
**ATTORNEY TO BE NOTICED**

**Counter Defendant**

**Baxalta Incorporated**  
*a Delaware corporation*

represented by **Jody Barillare**  
(See above for address)  
**LEAD ATTORNEY**  
**ATTORNEY TO BE NOTICED**

**Michael J. Abernathy**  
(See above for address)  
**LEAD ATTORNEY**  
**ATTORNEY TO BE NOTICED**

**Amanda S. Williamson**  
(See above for address)  
**ATTORNEY TO BE NOTICED**

**Amy Michele Dudash**  
(See above for address)  
*ATTORNEY TO BE NOTICED*

**Caroline Lourgous**  
(See above for address)  
*PRO HAC VICE*  
*ATTORNEY TO BE NOTICED*

**Colm F. Connolly**  
(See above for address)  
*TERMINATED: 11/07/2018*

**Jason C. White**  
(See above for address)  
*ATTORNEY TO BE NOTICED*

**Jennifer M. Dienes**  
(See above for address)  
*PRO HAC VICE*  
*ATTORNEY TO BE NOTICED*

**Jesse T. Dyer**  
(See above for address)  
*PRO HAC VICE*  
*ATTORNEY TO BE NOTICED*

**Karon N. Fowler**  
(See above for address)  
*ATTORNEY TO BE NOTICED*

**Maria E. Doukas**  
(See above for address)  
*ATTORNEY TO BE NOTICED*

Date Filed	#	Docket Text
05/04/2017	<u>1</u>	COMPLAINT FOR PATENT INFRINGEMENT AND DECLARATORY JUDGMENT OF PATENT INFRINGEMENT – filed with Jury Demand against Chugai Pharmaceutical Co., Ltd., Genentech, Inc. – Magistrate Consent Notice to Pltf. ( Filing fee \$ 400, receipt number 0311–2134340.) – filed by Baxalta Incorporated, Baxalta GmbH. (Attachments: # <u>1</u> Exhibit A, # <u>2</u> Exhibit B, # <u>3</u> Exhibit C, # <u>4</u> Exhibit D, # <u>5</u> Exhibit E, # <u>6</u> Exhibit F, # <u>7</u> Exhibit G, # <u>8</u> Exhibit H, # <u>9</u> Exhibit I, # <u>10</u> Exhibit J, # <u>11</u> Exhibit K, # <u>12</u> Exhibit L, # <u>13</u> Exhibit M, # <u>14</u> Civil Cover Sheet)(rwc) (Entered: 05/04/2017)
05/04/2017	<u>2</u>	Notice, Consent and Referral forms re: U.S. Magistrate Judge jurisdiction. (rwc) (Entered: 05/04/2017)
05/04/2017	<u>3</u>	Report to the Commissioner of Patents and Trademarks for Patent/Trademark Number(s) 7,033,590. (rwc) (Entered: 05/04/2017)
05/04/2017	<u>4</u>	Disclosure Statement pursuant to Rule 7.1: identifying Corporate Parent Shire plc for Baxalta Incorporated; Corporate Parent Baxalta Incorporated for Baxalta GmbH filed by Baxalta GmbH, Baxalta Incorporated. (rwc) (Entered: 05/04/2017)
05/04/2017		No Summons Issued. (rwc) (Entered: 05/04/2017)
05/10/2017		Case Assigned to Judge Gregory M. Sleet. Please include the initials of the Judge (GMS) after the case number on all documents filed. (rjb) (Entered: 05/10/2017)
05/11/2017		Summons Issued with Magistrate Consent Notice attached as to Genentech, Inc. on 5/11/2017. (sar) (Entered: 05/11/2017)
05/11/2017	<u>5</u>	MOTION for Pro Hac Vice Appearance of Attorney Michael J. Abernathy, Christopher J. Betti, Amanda S. Williamson, Jennifer M. Dienes, Maria E. Doukas and Jessica A. Stow – filed by

		Baxalta GmbH, Baxalta Incorporated. (Attachments: # <u>1</u> Michael Abernathy Certification by Counsel to be Admitted Pro Hac Vice, # <u>2</u> Christopher J. Betti Certification by Counsel to be Admitted Pro Hac Vice, # <u>3</u> Amanda Williamson Certification by Counsel to be Admitted Pro Hac Vice, # <u>4</u> Jennifer Dienes Certification by Counsel to be Admitted Pro Hac Vice, # <u>5</u> Maria Doukas Certification by Counsel to be Admitted Pro Hac Vice, # <u>6</u> Jessica Stow Certification by Counsel to be Admitted Pro Hac Vice)(Connolly, Colm) (Entered: 05/11/2017)
05/12/2017	<u>6</u>	Return of Service Executed by Baxalta Incorporated, Baxalta GmbH. Genentech, Inc. served on 5/11/2017, answer due 6/1/2017. (Connolly, Colm) (Entered: 05/12/2017)
05/16/2017		SO ORDERED – re <u>5</u> MOTION for Pro Hac Vice Appearance of Attorney Michael J. Abernathy, Christopher J. Betti, Amanda S. Williamson, Jennifer M. Dienes, Maria E. Doukas and Jessica A. Stow filed by Baxalta GmbH, Baxalta Incorporated. Ordered by Judge Gregory M. Sleet on 5/16/2017. (mdb) (Entered: 05/16/2017)
05/16/2017		Pro Hac Vice Attorney Amanda S. Williamson for Baxalta GmbH, Amanda S. Williamson for Baxalta Incorporated added for electronic noticing. Pursuant to Local Rule 83.5 (d)., Delaware counsel shall be the registered users of CM/ECF and shall be required to file all papers. (crb) (Entered: 05/16/2017)
05/22/2017		Pro Hac Vice Attorney Jennifer M. Dienes, Maria E. Doukas for Baxalta GmbH, Jennifer M. Dienes, Maria E. Doukas for Baxalta Incorporated added for electronic noticing. Pursuant to Local Rule 83.5 (d)., Delaware counsel shall be the registered users of CM/ECF and shall be required to file all papers. (crb) (Entered: 05/22/2017)
06/01/2017	<u>7</u>	STIPULATION TO EXTEND TIME by which defendant Genentech, Inc. must answer, move, or otherwise respond to the complaint to June 30, 2017, – filed by Genentech, Inc.. (Balick, Steven) (Entered: 06/01/2017)
06/01/2017		SO ORDERED – re <u>7</u> STIPULATION TO EXTEND TIME by which defendant Genentech, Inc. must answer, move, or otherwise respond to the complaint to June 30, 2017, filed by Genentech, Inc., Set/Reset Answer Deadlines: Genentech, Inc. answer due 6/30/2017. Ordered by Judge Gregory M. Sleet on 6/1/2017. (mdb) (Entered: 06/01/2017)
06/02/2017		Summons Issued with Magistrate Consent Notice attached as to Chugai Pharmaceutical Co., Ltd. on 6/2/2017. (nmg) (Entered: 06/02/2017)
06/20/2017	<u>8</u>	WAIVER OF SERVICE returned executed by Baxalta Incorporated, Baxalta GmbH: For Chugai Pharmaceutical Co., Ltd. waiver sent on 6/15/2017, answer due 9/13/2017. (Connolly, Colm) (Entered: 06/20/2017)
06/30/2017	<u>9</u>	ANSWER to <u>1</u> Complaint,, with Jury Demand , COUNTERCLAIM against Baxalta GmbH, Baxalta Incorporated by Genentech, Inc..(Balick, Steven) (Entered: 06/30/2017)
06/30/2017	<u>10</u>	Disclosure Statement pursuant to Rule 7.1: identifying Corporate Parent Roche Holdings, Inc. for Genentech, Inc. filed by Genentech, Inc.. (Balick, Steven) (Entered: 06/30/2017)
06/30/2017	<u>11</u>	MOTION for Pro Hac Vice Appearance of Attorney Nicholas P. Groombridge, Eric Alan Stone, Kenneth A. Gallo, Jennifer Gordon, Ph.D., and David E. Cole – filed by Genentech, Inc.. (Balick, Steven) (Entered: 06/30/2017)
06/30/2017	<u>12</u>	Amended Disclosure Statement pursuant to Rule 7.1: identifying Corporate Parent Roche Holdings, Inc. for Genentech, Inc. filed by Genentech, Inc.. (Mayo, Andrew) (Entered: 06/30/2017)
07/05/2017	<u>13</u>	ORDER REGARDING CASE MANAGEMENT IN CIVIL CASES. Signed by Judge Gregory M. Sleet on 7/5/2017. (asw) (Entered: 07/05/2017)
07/06/2017		SO ORDERED, re <u>11</u> MOTION for Pro Hac Vice Appearance of Attorney Nicholas P. Groombridge, Eric Alan Stone, Kenneth A. Gallo, Jennifer Gordon, Ph.D., and David E. Cole filed by Genentech, Inc. Signed by Judge Gregory M. Sleet on 7/6/2017. (asw) (Entered: 07/06/2017)
07/06/2017		Pro Hac Vice Attorney Eric A. Stone for Genentech, Inc. added for electronic noticing. Pursuant to Local Rule 83.5 (d)., Delaware counsel shall be the registered users of CM/ECF and shall be required to file all papers. (crb) (Entered: 07/06/2017)
07/10/2017		Pro Hac Vice Attorney Jennifer Gordon, David E. Cole, Kenneth A. Gallo for Genentech, Inc. added for electronic noticing. Pursuant to Local Rule 83.5 (d)., Delaware counsel shall be the



		registered users of CM/ECF and shall be required to file all papers. (nmg) (Entered: 07/10/2017)
07/21/2017	<u>14</u>	ANSWER to <u>9</u> Answer to Complaint, Counterclaim by Baxalta GmbH, Baxalta Incorporated.(Connolly, Colm) (Entered: 07/21/2017)
08/04/2017	<u>15</u>	Joint STATUS REPORT by Baxalta GmbH, Baxalta Incorporated. (Connolly, Colm) (Entered: 08/04/2017)
08/04/2017	<u>16</u>	PROPOSED ORDER – Scheduling Order by Baxalta GmbH, Baxalta Incorporated. (Connolly, Colm) (Entered: 08/04/2017)
08/04/2017	<u>17</u>	Amended STATUS REPORT (Submitted Jointly) by Baxalta GmbH, Baxalta Incorporated. (Connolly, Colm) Modified on 8/14/2017 (mdb). (Entered: 08/04/2017)
08/14/2017		Pro Hac Vice Attorney Nicolas P. Groombridge for Genentech, Inc. added for electronic noticing. Pursuant to Local Rule 83.5 (d)., Delaware counsel shall be the registered users of CM/ECF and shall be required to file all papers. (crb) (Entered: 08/14/2017)
09/13/2017	<u>18</u>	MOTION to Dismiss for Lack of Jurisdiction Over the Person – filed by Chugai Pharmaceutical Co., Ltd.. (Attachments: # <u>1</u> Text of Proposed Order)(Fry, David) (Entered: 09/13/2017)
09/13/2017	<u>19</u>	OPENING BRIEF in Support re <u>18</u> MOTION to Dismiss for Lack of Jurisdiction Over the Person filed by Chugai Pharmaceutical Co., Ltd..Answering Brief/Response due date per Local Rules is 9/27/2017. (Fry, David) (Entered: 09/13/2017)
09/13/2017	<u>20</u>	DECLARATION of Tetsuya Yamaguchi re <u>18</u> MOTION to Dismiss for Lack of Jurisdiction Over the Person by Chugai Pharmaceutical Co., Ltd.. (Fry, David) Modified on 9/14/2017 (mdb). (Entered: 09/13/2017)
09/13/2017	<u>21</u>	Disclosure Statement pursuant to Rule 7.1: identifying Corporate Parent Roche Holding Ltd. for Chugai Pharmaceutical Co., Ltd. filed by Chugai Pharmaceutical Co., Ltd.. (Fry, David) (Entered: 09/13/2017)
09/14/2017	<u>22</u>	MOTION for Pro Hac Vice Appearance of Attorney David C. Doyle and Brian M. Kramer – filed by Chugai Pharmaceutical Co., Ltd.. (Keller, Karen) (Entered: 09/14/2017)
09/15/2017		SO ORDERED – re <u>22</u> MOTION for Pro Hac Vice Appearance of Attorney David C. Doyle and Brian M. Kramer filed by Chugai Pharmaceutical Co., Ltd.. Ordered by Judge Gregory M. Sleet on 9/15/2017. (mdb) (Entered: 09/15/2017)
09/19/2017		ORAL ORDER re <u>17</u> Joint Status Report – Because defendant Chugai Pharmaceutical Co., Ltd. did not participate in the telephone conference prior to submitting the joint status report, defendant Chugai shall file a letter indicating their positions on the contents of the joint status report and proposed schedule within seven days of this order. Ordered by Judge Gregory M. Sleet on 9/19/2017. (mdb) (Entered: 09/19/2017)
09/20/2017		Pro Hac Vice Attorney David C. Doyle and Brian M. Kramer for Chugai Pharmaceutical Co., Ltd. added for electronic noticing. Pursuant to Local Rule 83.5 (d)., Delaware counsel shall be the registered users of CM/ECF and shall be required to file all papers. (ceg) (Entered: 09/20/2017)
09/26/2017	<u>23</u>	Letter to The Honorable Gregory M. Sleet from David M. Fry. (Fry, David) (Entered: 09/26/2017)
09/27/2017	<u>24</u>	ANSWERING BRIEF in Opposition re <u>18</u> MOTION to Dismiss for Lack of Jurisdiction Over the Person filed by Baxalta GmbH, Baxalta Incorporated.Reply Brief due date per Local Rules is 10/4/2017. (Connolly, Colm) (Entered: 09/27/2017)
09/27/2017	<u>25</u>	DECLARATION of Amanda S. Williamson re <u>24</u> Answering Brief in Opposition by Baxalta GmbH, Baxalta Incorporated. (Attachments: # <u>1</u> Exhibit 1, # <u>2</u> Exhibit 2, # <u>3</u> Exhibit 3, # <u>4</u> Exhibit 4, # <u>5</u> Exhibit 5, # <u>6</u> Exhibit 6, # <u>7</u> Exhibit 7, # <u>8</u> Exhibit 8, # <u>9</u> Exhibit 9, # <u>10</u> Exhibit 10, # <u>11</u> Exhibit 11, # <u>12</u> Exhibit 12)(Connolly, Colm) Modified on 10/2/2017 (mdb). (Entered: 09/27/2017)
09/29/2017	<u>26</u>	NOTICE OF SERVICE of Plaintiffs' Rule 26(a)(1) Initial Disclosures filed by Baxalta GmbH, Baxalta Incorporated.(Connolly, Colm) (Entered: 09/29/2017)
09/29/2017	<u>27</u>	NOTICE OF SERVICE of Defendant Genentech, Inc.'s Initial Disclosures Pursuant to Fed. R. Civ. P. 26(a)(1) filed by Genentech, Inc..(Mayo, Andrew) (Entered: 09/29/2017)

10/02/2017	<u>28</u>	NOTICE OF SERVICE of Defendant Chugai Pharmaceutical Co., Ltd.'s Initial Disclosures Pursuant to Federal Rule of Civil Procedure 26(a)(1) filed by Chugai Pharmaceutical Co., Ltd..(Keller, Karen) (Entered: 10/02/2017)
10/04/2017		ORAL ORDER re <u>17</u> Joint Status Report – The parties' proposed schedule is ADOPTED, with the following additions: The Markman Hearing (1/2 day) is set for 7/16/2018 at 9:30 AM in Courtroom 4A, the Final Pretrial Conference is set for 8/26/2019 at 10:00 AM in Courtroom 4A, and a 7–day Jury Trial is set for 9/9/2019 at 9:30 AM in Courtroom 4A. This case is referred to the Magistrate Judge for exploring ADR. The plaintiff shall file an amended scheduling order incorporating the full case schedule by 10/11/2017. Ordered by Judge Gregory M. Sleet on 10/4/2017. (mdb) (Entered: 10/04/2017)
10/04/2017	<u>29</u>	REPLY BRIEF re <u>18</u> MOTION to Dismiss for Lack of Jurisdiction Over the Person filed by Chugai Pharmaceutical Co., Ltd.. (Keller, Karen) (Entered: 10/04/2017)
10/11/2017	<u>30</u>	Letter to The Honorable Gregory M. Sleet from Colm F. Connolly regarding jointly submitting a scheduling order for the Court's consideration – re Oral Order,, (Attachments: # <u>1</u> Text of Proposed Order – Scheduling Order)(Connolly, Colm) (Entered: 10/11/2017)
10/12/2017		SO ORDERED – re <u>30</u> Scheduling Order: Case referred to the Magistrate Judge for the purpose of exploring ADR. (Motions for Joinder of Parties and Amended Pleadings due by 6/7/2018., Claim Construction Opening Briefs due by 5/11/2018., Claim Construction Answering Briefs due by 6/15/2018., A Markman Hearing (1/2 day) is set for 7/16/2018 at 09:30 AM in Courtroom 4A., Fact Discovery completed by 8/31/2018., Opening Expert Reports due by 10/5/2018., Rebuttal Expert Reports due by 11/9/2018., Reply Expert Reports due by 12/7/2018., Expert Discovery due by 1/31/2019., Opening Summary Judgment Letter Briefs due by 2/14/2019., Answering Letter Briefs due 2/28/2019., Reply Letter Briefs due 3/7/2019., Proposed Pretrial Order due by 8/16/2019., A Final Pretrial Conference is set for 8/26/2019 at 10:00 AM in Courtroom 4A., and a 7–day Jury Trial is set for 9/9/2019 at 09:30 AM in Courtroom 4A. See Scheduling Order ( <u>30</u> ) for more details). Ordered by Judge Gregory M. Sleet on 10/12/2017. (mdb) (Entered: 10/12/2017)
10/17/2017	<u>31</u>	NOTICE OF SERVICE of Plaintiffs' First Set of Requests for Production of Documents and Things (Nos. 1–107) filed by Baxalta GmbH, Baxalta Incorporated.(Connolly, Colm) (Entered: 10/17/2017)
10/23/2017		CASE REFERRED to Chief Magistrate Judge Mary Pat Thyng for Mediation. Please see Standing Order dated January 20, 2016, regarding disclosure of confidential ADR communications. A link to the standing order is provided here for your convenience at <a href="http://www.ded.uscourts.gov/general-orders/magistrate-judges-standing-order-adr-mediation">http://www.ded.uscourts.gov/general-orders/magistrate-judges-standing-order-adr-mediation</a> (cak) (Entered: 10/23/2017)
10/23/2017	<u>32</u>	STIPULATION Seeking to Extend the Deadline by which the Parties Must Submit a Proposed Protective Order, by Genentech, Inc.. (Balick, Steven) (Entered: 10/23/2017)
10/24/2017	<u>33</u>	ORDER Setting Teleconference: Plaintiffs' counsel to initiate the call. A Telephone Conference is set for 11/30/2017 at 11:00 AM Eastern Time before Judge Mary Pat Thyng to discuss ADR. Signed by Judge Mary Pat Thyng on 10/24/17. (cak) (Entered: 10/24/2017)
10/26/2017		SO ORDERED – re <u>32</u> Stipulation Seeking to Extend the Deadline by which the Parties Must Submit a Proposed Protective Order filed by Genentech, Inc.. Ordered by Judge Gregory M. Sleet on 10/26/2017. (mdb) (Entered: 10/26/2017)
11/02/2017	<u>34</u>	STIPULATION TO EXTEND TIME to Submit a Proposed Protective Order to November 9, 2017 – filed by Chugai Pharmaceutical Co., Ltd.. (Shaw, John) (Entered: 11/02/2017)
11/02/2017		SO ORDERED – re <u>34</u> STIPULATION TO EXTEND TIME to Submit a Proposed Protective Order to November 9, 2017 filed by Chugai Pharmaceutical Co., Ltd.. Ordered by Judge Gregory M. Sleet on 11/2/2017. (mdb) (Entered: 11/02/2017)
11/02/2017	<u>35</u>	NOTICE OF SERVICE of Genentech, Inc.'s First Set of Requests for the Production of Documents and Things to Baxalta Incorporated and Baxalta GmbH filed by Genentech, Inc..(Mayo, Andrew) (Entered: 11/02/2017)
11/03/2017	<u>36</u>	NOTICE OF SERVICE of Defendant Genentech, Inc.'s Initial Disclosures Pursuant to Section 3 of the Default Standard for Discovery filed by Genentech, Inc..(Balick, Steven) (Entered: 11/03/2017)

11/06/2017	<u>37</u>	NOTICE OF SERVICE of Chugai Pharmaceutical Co., Ltd.'s Initial Disclosures Pursuant to Paragraph 3 of the Default Standard for Discovery filed by Chugai Pharmaceutical Co., Ltd..(Keller, Karen) (Entered: 11/06/2017)
11/09/2017	<u>38</u>	PROPOSED ORDER – Stipulated Protective Order by Baxalta GmbH, Baxalta Incorporated. (Connolly, Colm) (Entered: 11/09/2017)
11/13/2017		SO ORDERED – re <u>38</u> (Proposed) Stipulated Protective Order filed by Baxalta GmbH, Baxalta Incorporated. Ordered by Judge Gregory M. Sleet on 11/13/2017. (mdb) (Entered: 11/13/2017)
11/17/2017	<u>39</u>	NOTICE OF SERVICE of Plaintiffs' Initial Disclosure of Infringement Contentions Pursuant to October 11, 2017 Scheduling Order filed by Baxalta GmbH, Baxalta Incorporated.(Connolly, Colm) (Entered: 11/17/2017)
12/12/2017	<u>40</u>	NOTICE of Appearance by Amy Michele Dudash on behalf of All Plaintiffs (Dudash, Amy) (Entered: 12/12/2017)
12/14/2017	<u>41</u>	MOTION for Preliminary Injunction – filed by Baxalta GmbH, Baxalta Incorporated. (Connolly, Colm) (Entered: 12/14/2017)
12/14/2017	<u>42</u>	[SEALED] MEMORANDUM in Support re <u>41</u> MOTION for Preliminary Injunction filed by Baxalta GmbH, Baxalta Incorporated. Answering Brief/Response due date per Local Rules is 12/28/2017. (Attachments: # <u>1</u> Text of Proposed Order)(Connolly, Colm) (Entered: 12/14/2017)
12/14/2017	<u>43</u>	[SEALED] DECLARATION re <u>42</u> MEMORANDUM in Support of Plaintiffs' Memorandum in Support of Their Motion for Preliminary Injunction by Baxalta GmbH, Baxalta Incorporated. (Attachments: # <u>1</u> Exhibit Exhibits M, EE, JJ and PP that are Sealed)(Connolly, Colm) Modified on 12/15/2017 (mdb). (Entered: 12/14/2017)
12/14/2017	<u>44</u>	EXHIBIT A re <u>43</u> Declaration by Baxalta GmbH, Baxalta Incorporated. (Attachments: # <u>1</u> Exhibit B, # <u>2</u> Exhibit C, # <u>3</u> Exhibit D, # <u>4</u> Exhibit E, # <u>5</u> Exhibit F, # <u>6</u> Exhibit G, # <u>7</u> Exhibit H, # <u>8</u> Exhibit I, # <u>9</u> Exhibit J, # <u>10</u> Exhibit K, # <u>11</u> Exhibit L, # <u>12</u> Exhibit N, # <u>13</u> Exhibit O, # <u>14</u> Exhibit P, # <u>15</u> Exhibit Q, # <u>16</u> Exhibit R, # <u>17</u> Exhibit S)(Connolly, Colm) Modified on 12/15/2017 (mdb). (Entered: 12/14/2017)
12/14/2017	<u>45</u>	EXHIBIT T re <u>44</u> Exhibit to a Document, <u>43</u> Declaration by Baxalta GmbH, Baxalta Incorporated. (Attachments: # <u>1</u> Exhibit U, # <u>2</u> Exhibit V, # <u>3</u> Exhibit W, # <u>4</u> Exhibit X, # <u>5</u> Exhibit Y, # <u>6</u> Exhibit Z, # <u>7</u> Exhibit AA, # <u>8</u> Exhibit BB, # <u>9</u> Exhibit CC, # <u>10</u> Exhibit DD, # <u>11</u> Exhibit FF, # <u>12</u> Exhibit GG, # <u>13</u> Exhibit HH, # <u>14</u> Exhibit II, # <u>15</u> Exhibit KK, # <u>16</u> Exhibit LL, # <u>17</u> Exhibit MM, # <u>18</u> Exhibit NN, # <u>19</u> Exhibit OO, # <u>20</u> Exhibit QQ, # <u>21</u> Exhibit RR, # <u>22</u> Exhibit SS, # <u>23</u> Exhibit TT, # <u>24</u> Exhibit UU, # <u>25</u> Exhibit VV, # <u>26</u> Exhibit WW)(Connolly, Colm) Modified on 12/15/2017 (mdb). (Entered: 12/14/2017)
12/14/2017	<u>46</u>	DECLARATION of Dr. Louis M. Aledort re <u>42</u> MEMORANDUM in Support by Baxalta GmbH, Baxalta Incorporated. (Attachments: # <u>1</u> Exhibit A, # <u>2</u> Exhibit B, # <u>3</u> Exhibit C, # <u>4</u> Exhibit D, # <u>5</u> Exhibit E, # <u>6</u> Exhibit F, # <u>7</u> Exhibit G, # <u>8</u> Exhibit H, # <u>9</u> Exhibit I, # <u>10</u> Exhibit J, # <u>11</u> Exhibit K, # <u>12</u> Exhibit L, # <u>13</u> Exhibit M, # <u>14</u> Exhibit N, # <u>15</u> Exhibit O)(Connolly, Colm) Modified on 12/15/2017 (mdb). (Entered: 12/14/2017)
12/14/2017	<u>47</u>	DECLARATION of Krishnaswamy, Ph.D. re <u>42</u> MEMORANDUM in Support by Baxalta GmbH, Baxalta Incorporated. (Attachments: # <u>1</u> Exhibit A, # <u>2</u> Exhibit B, # <u>3</u> Exhibit C, # <u>4</u> Exhibit D, # <u>5</u> Exhibit E, # <u>6</u> Exhibit F, # <u>7</u> Exhibit H, # <u>8</u> Exhibit I, # <u>9</u> Exhibit K, # <u>10</u> Exhibit L, # <u>11</u> Exhibit M, # <u>12</u> Exhibit N, # <u>13</u> Exhibit O, # <u>14</u> Exhibit P, # <u>15</u> Exhibit Q, # <u>16</u> Exhibit R, # <u>17</u> Exhibit S, # <u>18</u> Exhibit T, # <u>19</u> Exhibit U, # <u>20</u> Exhibit V, # <u>21</u> Exhibit W, # <u>22</u> Exhibit X, # <u>23</u> Exhibit Y, # <u>24</u> Exhibit Z, # <u>25</u> Exhibit AA)(Connolly, Colm) Modified on 12/15/2017 (mdb). (Entered: 12/14/2017)
12/14/2017	<u>48</u>	DECLARATION of Anthony A. Kossiakoff, Ph.D. re <u>42</u> MEMORANDUM in Support by Baxalta GmbH, Baxalta Incorporated. (Attachments: # <u>1</u> Exhibit A, # <u>2</u> Exhibit B, # <u>3</u> Exhibit C, # <u>4</u> Exhibit D, # <u>5</u> Exhibit E, # <u>6</u> Exhibit F, # <u>7</u> Exhibit G, # <u>8</u> Exhibit H, # <u>9</u> Exhibit I, # <u>10</u> Exhibit J, # <u>11</u> Exhibit K, # <u>12</u> Exhibit L, # <u>13</u> Exhibit M, # <u>14</u> Exhibit N, # <u>15</u> Exhibit O, # <u>16</u> Exhibit P, # <u>17</u> Exhibit Q, # <u>18</u> Exhibit R, # <u>19</u> Exhibit S, # <u>20</u> Exhibit T, # <u>21</u> Exhibit U, # <u>22</u> Exhibit V, # <u>23</u> Exhibit W, # <u>24</u> Exhibit X, # <u>25</u> Exhibit Y, # <u>26</u> Exhibit Z, # <u>27</u> Exhibit AA, # <u>28</u> Exhibit BB, # <u>29</u> Exhibit CC, # <u>30</u> Exhibit DD, # <u>31</u> Exhibit EE, # <u>32</u> Exhibit FF, # <u>33</u> Exhibit GG, # <u>34</u> Exhibit HH)(Connolly, Colm) Modified on 12/15/2017 (mdb). (Entered: 12/14/2017)

12/14/2017	<u>49</u>	DECLARATION of Juan Carlos Almagro, Ph.D. re <u>42</u> MEMORANDUM in Support by Baxalta GmbH, Baxalta Incorporated. (Attachments: # <u>1</u> Exhibit A, # <u>2</u> Exhibit B, # <u>3</u> Exhibit C, # <u>4</u> Exhibit D, # <u>5</u> Exhibit E, # <u>6</u> Exhibit F, # <u>7</u> Exhibit G, # <u>8</u> Exhibit H, # <u>9</u> Exhibit I, # <u>10</u> Exhibit J, # <u>11</u> Exhibit K, # <u>12</u> Exhibit L)(Connolly, Colm) Modified on 12/15/2017 (mdb). (Entered: 12/14/2017)
12/15/2017	<u>50</u>	NOTICE OF SERVICE of Genentech's Preliminary Invalidity Contentions filed by Genentech, Inc..(Mayo, Andrew) (Entered: 12/15/2017)
12/18/2017		ORAL ORDER re <u>41</u> Motion for Preliminary Injunction – A Telephone Conference is set for today, December 18, 2017 at 2:00 PM (EST). The plaintiffs shall arrange the call and contact Chambers at 302–573–4559 once all parties are on the line. Ordered by Judge Gregory M. Sleet on 12/18/2017. (mdb) (Entered: 12/18/2017)
12/18/2017	<u>51</u>	NOTICE of Appearance by Jody Barillare on behalf of All Plaintiffs (Barillare, Jody) (Entered: 12/18/2017)
12/18/2017		Minute Entry for proceedings held before Judge Gregory M. Sleet – Telephone Conference held on 12/18/2017, (A Status Telephone Conference regarding the Motion for Preliminary Injunction ( <u>41</u> ) is set for Friday, December 22, 2017 at 3:00 PM. Counsel for plaintiffs shall arrange the call and contact Chambers at 302–573–4559 once all parties are on the line. (Court Reporter Kevin Maurer.) (mdb) (Entered: 12/18/2017)
12/18/2017	<u>52</u>	NOTICE OF SERVICE of Defendant Chugai Pharmaceutical Co., Ltd.'s Preliminary Invalidity Contentions filed by Chugai Pharmaceutical Co., Ltd..(Fry, David) (Entered: 12/18/2017)
12/18/2017	<u>53</u>	NOTICE OF SERVICE of Genentech's Objections and Responses to Baxalta's First Set of Requests for Production filed by Genentech, Inc..(Mayo, Andrew) (Entered: 12/18/2017)
12/19/2017		ORAL ORDER CANCELLING TELECONFERENCE: The Status Telephone Conference regarding <u>41</u> Motion for Preliminary Injunction previously set for Friday, December 22, 2017 at 3:00 PM is CANCELLED. The parties shall submit a Stipulation with a proposed schedule and include a date the parties are available to attend a hearing on the Motion for Preliminary Injunction. If the parties are unable to agree on a proposed schedule, the court shall set a Telephone Conference on Friday, January 5, 2018 at 10:00 AM. The parties shall contact chambers if a Teleconference is not necessary. Ordered by Judge Gregory M. Sleet on 12/19/2017. (asw) (Entered: 12/19/2017)
12/20/2017		Set/Reset Hearings: A Telephone Conference re: Motion for Preliminary Injunction is TENTATIVELY set for 1/5/2018 at 10:00 AM before Judge Gregory M. Sleet. (asw) (Entered: 12/20/2017)
12/20/2017	<u>54</u>	NOTICE OF SERVICE of Defendant Chugai Pharmaceutical Co., Ltd.'s Responses to Plaintiffs' First Set of Requests for Production of Documents and Things (Nos. 1–107) filed by Chugai Pharmaceutical Co., Ltd..(Fry, David) (Entered: 12/20/2017)
12/20/2017		Pro Hac Vice Attorney Michael J. Abernathy and Christopher J Betti for Baxalta Incorporated added for electronic noticing. Pursuant to Local Rule 83.5 (d)., Delaware counsel shall be the registered users of CM/ECF and shall be required to file all papers. (lmm) (Entered: 12/20/2017)
12/21/2017	<u>55</u>	REDACTED VERSION of <u>42</u> MEMORANDUM in Support of its Motion for Preliminary Injunction by Baxalta GmbH, Baxalta Incorporated. (Connolly, Colm) Modified on 12/21/2017 (mdb). (Entered: 12/21/2017)
12/21/2017	<u>56</u>	REDACTED VERSION of [Proposed] Preliminary Injunction Order by Baxalta GmbH, Baxalta Incorporated. (Connolly, Colm) Modified on 12/21/2017 (mdb). (Entered: 12/21/2017)
12/21/2017	<u>57</u>	REDACTED VERSION of <u>43</u> Declaration of W. Christopher Bakewell Regarding Irreparable Harm and Balance of Hardships in Support of Plaintiffs' Memorandum in Support of Their Motion for Preliminary Injunction by Baxalta GmbH, Baxalta Incorporated. (Attachments: # <u>1</u> Public Versions of Exhibits M, EE, JJ, and PP)(Connolly, Colm) Modified on 12/21/2017 (mdb). (Entered: 12/21/2017)
12/22/2017	<u>58</u>	Official Transcript of Telephone Conference held on 12 18 17 before Judge Sleet. Court Reporter/Transcriber Maurer. Transcript may be viewed at the court public terminal or purchased through the Court Reporter/Transcriber before the deadline for Release of Transcript Restriction. After that date it may be obtained through PACER. Redaction Request due 1/12/2018. Redacted Transcript Deadline set for 1/22/2018. Release of Transcript Restriction set for 3/22/2018. (kjm) (Entered: 12/22/2017)

01/02/2018	<u>59</u>	NOTICE OF SERVICE of Baxalta Incorporated and Baxalta GMBH Responses and Objections to Genentech, Inc.'s First Set of Requests for the Production of Documents and Things filed by Baxalta GmbH, Baxalta Incorporated.(Dudash, Amy) (Entered: 01/02/2018)
01/04/2018	<u>60</u>	Joint STIPULATION Regarding Scheduling for Plaintiffs' Motion for Preliminary Injunction re Oral Order,, Set/Reset Hearings by Baxalta Incorporated. (Dudash, Amy) (Entered: 01/04/2018)
01/05/2018	<u>61</u>	SO ORDERED – re <u>60</u> Stipulation and Proposed Scheduling Order re Motion for Preliminary Injunction ( <u>41</u> ): Answering Brief due 4/6/2018, Reply Brief due 4/24/2018, Oral Argument is set for 5/24/2018 at 09:00 AM in Courtroom 4A. See stipulation for more details. The January 5, 2018 telephone conference is CANCELLED. Signed by Judge Gregory M. Sleet on 1/5/2018. (mdb) (Entered: 01/05/2018)
01/05/2018	<u>62</u>	NOTICE OF SERVICE of Plaintiffs' First Set of Expedited Requests for Production of Documents and Things to Defendant (Nos. 1–13) Relating to the Motion for Preliminary Injunction filed by Baxalta GmbH, Baxalta Incorporated.(Dudash, Amy) (Entered: 01/05/2018)
01/05/2018	<u>63</u>	NOTICE OF SERVICE of Genentech's Discovery Requests Related to Plaintiffs' Motion for a Preliminary Injunction filed by Genentech, Inc..(Mayo, Andrew) (Entered: 01/05/2018)
01/09/2018	<u>64</u>	ORAL ORDER Setting Teleconference: Plaintiffs' counsel to initiate the call. A Telephone Conference is set for 6/29/2018 at 11:00 AM Eastern Time before Judge Mary Pat Thyng to discuss the status of the case, the parties to be involved in mediation in March 2019 and submissions. Ordered by Judge Mary Pat Thyng on 1/9/18. (cak) (Entered: 01/09/2018)
01/09/2018	<u>65</u>	MOTION for Pro Hac Vice Appearance of Attorney Caroline S. Lourgos – filed by Baxalta GmbH, Baxalta Incorporated. (Dudash, Amy) (Entered: 01/09/2018)
01/10/2018		SO ORDERED – re <u>65</u> MOTION for Pro Hac Vice Appearance of Attorney Caroline S. Lourgos filed by Baxalta GmbH, Baxalta Incorporated. Ordered by Judge Gregory M. Sleet on 1/10/2018. (mdb) (Entered: 01/10/2018)
01/10/2018		Pro Hac Vice Attorney Caroline Lourgos for Baxalta GmbH,Baxalta Incorporated added for electronic noticing. Pursuant to Local Rule 83.5 (d), Delaware counsel shall be the registered users of CM/ECF and shall be required to file all papers. (crb) (Entered: 01/10/2018)
01/10/2018	<u>66</u>	NOTICE OF SERVICE of Baxalta Incorporated and Baxalta GmbH Responses and Objections to Genentech, Inc.'s Discovery Requests Related to Plaintiffs' Motion for a Preliminary Injunction filed by Baxalta GmbH, Baxalta Incorporated.(Dudash, Amy) (Entered: 01/10/2018)
01/10/2018	<u>67</u>	NOTICE OF SERVICE of Genentech's Objections and Responses to Plaintiffs' Expedited Requests for Production filed by Genentech, Inc..(Balick, Steven) (Entered: 01/10/2018)
01/12/2018	<u>68</u>	MOTION for Pro Hac Vice Appearance of Attorney Sanjay K. Murthy – filed by Baxalta GmbH, Baxalta Incorporated. (Dudash, Amy) (Entered: 01/12/2018)
01/16/2018		SO ORDERED – re <u>68</u> MOTION for Pro Hac Vice Appearance of Attorney Sanjay K. Murthy filed by Baxalta GmbH, Baxalta Incorporated. Ordered by Judge Gregory M. Sleet on 1/16/2018. (mdb) (Entered: 01/16/2018)
01/17/2018		Pro Hac Vice Attorney Sanjay K. Murthy for Baxalta GmbH added for electronic noticing. Pursuant to Local Rule 83.5 (d), Delaware counsel shall be the registered users of CM/ECF and shall be required to file all papers. (ceg) (Entered: 01/17/2018)
01/17/2018	<u>69</u>	NOTICE OF SERVICE of Plaintiffs' First Set of Interrogatories (1–20) filed by Baxalta GmbH, Baxalta Incorporated.(Dudash, Amy) (Entered: 01/17/2018)
01/19/2018	<u>70</u>	NOTICE OF SERVICE of Plaintiffs' List of Claim Terms in Need of Construction filed by Baxalta GmbH, Baxalta Incorporated.(Dudash, Amy) (Entered: 01/19/2018)
01/19/2018	<u>71</u>	NOTICE to Take Deposition of Genentech, Inc. and Chugai Pharmaceutical Co., Ltd. on mutually agreeable date in accordance with the scheduling order filed by Baxalta GmbH, Baxalta Incorporated.(Dudash, Amy) (Entered: 01/19/2018)
01/19/2018	<u>72</u>	NOTICE OF SERVICE of Genentech's Identification of Claim Terms that Need to be Construed filed by Genentech, Inc..(Mayo, Andrew) (Entered: 01/19/2018)
01/22/2018	<u>73</u>	NOTICE OF SERVICE of (1) Chugai Pharmaceutical Co., Ltd.'s Identification of Claim Terms that Need to be Construed filed by Chugai Pharmaceutical Co., Ltd..(Fry, David) (Entered: 01/22/2018)

01/31/2018	<u>74</u>	NOTICE OF SERVICE of Baxalta Incorporated and Baxalta GmbH's Supplemental Responses and Objections to Genentech, Inc.'s Interrogatories Nos. 1–2 and Requests for Admission Nos. 3, 4, 7 and 8 filed by Baxalta GmbH, Baxalta Incorporated.(Dudash, Amy) (Entered: 01/31/2018)
02/06/2018	<u>75</u>	MOTION for Pro Hac Vice Appearance of Attorney Jesse T. Dyer – filed by Baxalta GmbH, Baxalta Incorporated. (Dudash, Amy) (Entered: 02/06/2018)
02/06/2018	<u>76</u>	NOTICE OF SERVICE of Genentech, Inc.'s Notice of Subpoenas to Kilpatrick Townsend & Stockton LLP filed by Genentech, Inc..(Mayo, Andrew) (Entered: 02/06/2018)
02/06/2018		ORAL ORDER – A Discovery Dispute Telephone Conference (#1) is set for Monday, February 12, 2018 at 10:00 AM (EST). Counsel for the plaintiffs shall arrange the call and contact Chambers at 302–573–4559 once all parties are on the line. A non–argumentative Joint Agenda Letter (see www.ded.uscourts.gov for an example) is due by noon on Thursday, February 8, 2018. Ordered by Judge Gregory M. Sleet on 2/6/2018. (mdb) (Entered: 02/06/2018)
02/07/2018	<u>77</u>	NOTICE OF SERVICE of Genentech, Inc.'s Second Set of Requests for the Production of Documents and Things to Baxalta Incorporated and Baxalta GmbH (Nos. 99–101) and Genentech, Inc.'s First Set of Interrogatories (Nos. 3–11) filed by Genentech, Inc..(Mayo, Andrew) (Entered: 02/07/2018)
02/07/2018		SO ORDERED – re <u>75</u> MOTION for Pro Hac Vice Appearance of Attorney Jesse T. Dyer filed by Baxalta GmbH, Baxalta Incorporated. Ordered by Judge Gregory M. Sleet on 2/7/2018. (mdb) (Entered: 02/07/2018)
02/08/2018		Pro Hac Vice Attorney Jesse T. Dyer for Baxalta GmbH,Baxalta Incorporated added for electronic noticing. Pursuant to Local Rule 83.5 (d)., Delaware counsel shall be the registered users of CM/ECF and shall be required to file all papers. (crb) (Entered: 02/08/2018)
02/08/2018	<u>78</u>	NOTICE OF SERVICE of Genentech, Inc.'s Notice of Subpoena to Haematologic Technologies, Inc. filed by Genentech, Inc..(Mayo, Andrew) (Entered: 02/08/2018)
02/08/2018	<u>79</u>	Letter to The Honorable Gregory M. Sleet from Steven J. Balick regarding the Parties' Joint Agenda for the Teleconference on February 12, 2018 at 10:00 a.m.. (Balick, Steven) (Entered: 02/08/2018)
02/08/2018	<u>80</u>	NOTICE OF SERVICE of Notice of Subpoenas to Thomas A. Larmondra filed by Genentech, Inc..(Mayo, Andrew) (Entered: 02/08/2018)
02/08/2018	<u>81</u>	MOTION for Pro Hac Vice Appearance of Attorney Stephen D. Keane – filed by Chugai Pharmaceutical Co., Ltd.. (Keller, Karen) (Entered: 02/08/2018)
02/09/2018		SO ORDERED – re <u>81</u> MOTION for Pro Hac Vice Appearance of Attorney Stephen D. Keane filed by Chugai Pharmaceutical Co., Ltd. Ordered by Judge Gregory M. Sleet on 2/9/2018. (mdb) (Entered: 02/09/2018)
02/09/2018	<u>82</u>	NOTICE OF SERVICE of Genentech, Inc.'s Notice of Subpoenas to Shire LLC filed by Genentech, Inc..(Balick, Steven) (Entered: 02/09/2018)
02/09/2018	<u>83</u>	NOTICE OF SERVICE of Genentech, Inc.'s Notice of Subpoenas to Shire US Inc. filed by Genentech, Inc..(Balick, Steven) (Entered: 02/09/2018)
02/12/2018		Minute Entry for proceedings held before Judge Gregory M. Sleet – Telephone Conference held on 2/12/2018. Discussion on the disputed item listed in the Joint Agenda submission; extending the briefing schedule regarding the preliminary injunction and amending the schedule. The court will set a 2–day Hearing Re: Preliminary Injunction on 6/13/2018 at 9:30 AM through 6/14/2018. The court ordered the parties to file an Amended Scheduling Order as discussed during the Teleconference. (Court Reporter: Kevin Maurer.) (asw) (Entered: 02/12/2018)
02/12/2018		Set/Reset Hearings: An Oral Argument (2 days) re <u>41</u> Motion for Preliminary Injunction is set for 6/13/2018 at 9:30 AM in Courtroom 4A before Judge Gregory M. Sleet. (mdb) (Entered: 02/12/2018)
02/12/2018		Pro Hac Vice Attorney Stephen D. Keane for Chugai Pharmaceutical Co., Ltd. added for electronic noticing. Pursuant to Local Rule 83.5 (d)., Delaware counsel shall be the registered users of CM/ECF and shall be required to file all papers. (ceg) (Entered: 02/12/2018)
02/16/2018	<u>84</u>	NOTICE OF SERVICE of Genentech, Inc.'s Objections and Responses to Baxalta's First Set of Interrogatories (Nos. 1–20) filed by Genentech, Inc..(Mayo, Andrew) (Entered: 02/16/2018)

02/19/2018	<u>85</u>	NOTICE OF SERVICE of (1) Defendant Chugai Pharmaceutical Co., Ltd.'s Responses to Plaintiffs' First Set of Interrogatories (Nos. 1–20) filed by Chugai Pharmaceutical Co., Ltd..(Keller, Karen) (Entered: 02/19/2018)
02/23/2018	<u>86</u>	Official Transcript of Teleconference held on 2/12/18 before Judge Sleet. Court Reporter/Transcriber Maurer. Transcript may be viewed at the court public terminal or purchased through the Court Reporter/Transcriber before the deadline for Release of Transcript Restriction. After that date it may be obtained through PACER. Redaction Request due 3/16/2018. Redacted Transcript Deadline set for 3/26/2018. Release of Transcript Restriction set for 5/24/2018. (kjm) (Entered: 02/23/2018)
02/23/2018	<u>87</u>	NOTICE OF SERVICE of Defendant Genentech, Inc.'s Preliminary Identification of Claim Terms and Proposed Constructions filed by Genentech, Inc..(Mayo, Andrew) (Entered: 02/23/2018)
02/23/2018	<u>88</u>	NOTICE OF SERVICE of Plaintiffs' Proposed Claim Constructions filed by Baxalta GmbH, Baxalta Incorporated.(Dudash, Amy) (Entered: 02/23/2018)
02/26/2018	<u>89</u>	NOTICE OF SERVICE of (1) Defendant Chugai Pharmaceutical Co., Ltd.'s Preliminary Identification of Claim Terms and Proposed Constructions filed by Chugai Pharmaceutical Co., Ltd..(Keller, Karen) (Entered: 02/26/2018)
02/28/2018		Case Reassigned to Judge Leonard P. Stark. Please include the initials of the Judge (LPS) after the case number on all documents filed. (rjb) (Entered: 02/28/2018)
02/28/2018	<u>90</u>	NOTICE OF SERVICE of Defendant Genentech's Notice of Deposition of Plaintiffs Baxalta Incorporated and Baxalta GmbH Related to Plaintiffs' Motion for Preliminary Injunction filed by Genentech, Inc..(Mayo, Andrew) (Entered: 02/28/2018)
03/01/2018	<u>91</u>	NOTICE OF SERVICE of Non–Parties Shire LLC, Shire US, Inc., and Thomas A. Larmondra's Objections and Responses to Genentech, Inc.'s Subpoenas re <u>83</u> Notice of Service, <u>80</u> Notice of Service, <u>82</u> Notice of Service filed by Baxalta GmbH, Baxalta Incorporated.(Dudash, Amy) (Entered: 03/01/2018)
03/02/2018	<u>92</u>	MOTION for Pro Hac Vice Appearance of Attorney James V. Razick – filed by Genentech, Inc.. (Mayo, Andrew) (Entered: 03/02/2018)
03/02/2018	<u>93</u>	Joint STIPULATION Regarding Revised Schedule for Plaintiffs' Motion for Preliminary Injunction by Genentech, Inc.. (Mayo, Andrew) (Entered: 03/02/2018)
03/05/2018		SO ORDERED, re <u>92</u> MOTION for Pro Hac Vice Appearance of Attorney James V. Razick filed by Genentech, Inc. Signed by Judge Leonard P. Stark on 3/5/18. (ntl) (Entered: 03/05/2018)
03/05/2018	<u>94</u>	NOTICE OF SERVICE of Baxalta Incorporated and Baxalta GmbH's Responses and Objections to Genentech's Notice of Deposition re <u>90</u> Notice of Service filed by Baxalta GmbH, Baxalta Incorporated.(Dudash, Amy) (Entered: 03/05/2018)
03/09/2018	<u>95</u>	SO ORDERED, re <u>93</u> Amended Stipulation and Scheduling Order — the Markman Hearing is rescheduled for 8/2/2018 at 10:00 AM in Courtroom 6B. Signed by Judge Leonard P. Stark on 3/9/18. (ntl) (Entered: 03/09/2018)
03/09/2018	<u>96</u>	NOTICE OF SERVICE of Baxalta Incorporated and Baxalta GmbH Responses and Objections to Genentech, Inc.'s Second Set of Interrogatories and Baxalta Incorporated and Baxalta GmbH's Responses and Objections to Genentech, Inc.'s Second Set of Requests for the Production of Documents and Things filed by Baxalta GmbH, Baxalta Incorporated.(Dudash, Amy) (Entered: 03/09/2018)
03/23/2018	<u>97</u>	NOTICE OF SERVICE of Baxalta Incorporated and Baxalta GmbH's First Set of Requests for Admission to Chugai Pharmaceutical Co., Ltd. (Nos. 1–6) and Baxalta Incorporated and Baxalta GmbH's First Set of Requests for Admission to Genentech, Inc. (Nos. 1–3) filed by Baxalta GmbH, Baxalta Incorporated.(Dudash, Amy) (Entered: 03/23/2018)
03/23/2018	<u>98</u>	DECLARATION of Gallia Levy, M.D., Ph.D. in Opposition to Plaintiffs' Motion for a Preliminary Injunction by Genentech, Inc.. (Mayo, Andrew) (Entered: 03/23/2018)
03/23/2018	<u>99</u>	DECLARATION of Harvey Liverman in Opposition to Plaintiffs' Motion for a Preliminary Injunction by Genentech, Inc.. (Mayo, Andrew) (Entered: 03/23/2018)

03/23/2018	<u>100</u>	[SEALED] DECLARATION of Gina Chapman in Opposition to Plaintiffs' Motion for a Preliminary Injunction by Genentech, Inc.. (Mayo, Andrew) (Entered: 03/23/2018)
03/23/2018	<u>101</u>	REDACTED VERSION of <u>100</u> Declaration of Gina Chapman in Opposition to Plaintiffs' Motion for a Preliminary Injunction by Genentech, Inc.. (Mayo, Andrew) (Entered: 03/23/2018)
03/23/2018	<u>102</u>	[SEALED] DECLARATION of Greg Hogan in Opposition to Plaintiffs' Motion for a Preliminary Injunction by Genentech, Inc.. (Mayo, Andrew) (Entered: 03/23/2018)
03/23/2018	<u>103</u>	REDACTED VERSION of <u>102</u> Declaration of Greg Hogan in Opposition to Plaintiffs' Motion for a Preliminary Injunction by Genentech, Inc.. (Mayo, Andrew) (Entered: 03/23/2018)
03/23/2018	<u>104</u>	[SEALED] DECLARATION of Chelsey Serrano in Opposition to Plaintiffs' Motion for a Preliminary Injunction by Genentech, Inc.. (Mayo, Andrew) (Entered: 03/23/2018)
03/23/2018	<u>105</u>	REDACTED VERSION of <u>104</u> Declaration of Chelsey Serrano in Opposition to Plaintiffs' Motion for a Preliminary Injunction by Genentech, Inc.. (Mayo, Andrew) (Entered: 03/23/2018)
03/29/2018	<u>106</u>	NOTICE of Asserted Claim In Baxalta's Opening Memorandum In Support of Its Motion for a Preliminary Injunction by Baxalta GmbH, Baxalta Incorporated re <u>42</u> MEMORANDUM in Support, (Dudash, Amy) (Entered: 03/29/2018)
03/29/2018	<u>107</u>	Amended EXHIBIT re <u>46</u> Declaration, Amended Exhibit B to Declaration of Dr. Aledort by Baxalta GmbH, Baxalta Incorporated. (Dudash, Amy) (Entered: 03/29/2018)
03/30/2018	<u>108</u>	[SEALED] DECLARATION of Jerry A. Hausman, Ph.D. in Opposition to Plaintiffs' Motion for a Preliminary Injunction by Genentech, Inc.. (Mayo, Andrew) (Entered: 03/30/2018)
03/30/2018	<u>109</u>	[SEALED] DECLARATION of Michael U. Callaghan, M.D. In Support of Genentech, Inc.'s Opposition to Plaintiffs' Motion for a Preliminary Injunction by Genentech, Inc.. (Mayo, Andrew) (Entered: 03/30/2018)
03/30/2018	<u>110</u>	[SEALED] DECLARATION of Guy A. Young, M.D. in Opposition to Plaintiffs' Motion for a Preliminary Injunction by Genentech, Inc.. (Mayo, Andrew) (Entered: 03/30/2018)
03/30/2018	<u>111</u>	[SEALED] DECLARATION of John P. Sheehan, M.D. in Support of Genentech's Opposition to Baxalta's Motion for a Preliminary Injunction by Genentech, Inc.. (Attachments: # <u>1</u> Exhibit A, # <u>2</u> Exhibit B, # <u>3</u> Exhibit C, # <u>4</u> Exhibit D, # <u>5</u> Exhibit E, # <u>6</u> Exhibit F, # <u>7</u> Exhibit G, # <u>8</u> Exhibit H, # <u>9</u> Exhibit I, # <u>10</u> Exhibit J, # <u>11</u> Exhibit K, # <u>12</u> Exhibit L, # <u>13</u> Exhibit M, # <u>14</u> Exhibit N, # <u>15</u> Exhibit O, # <u>16</u> Exhibit P, # <u>17</u> Exhibit Q, # <u>18</u> Exhibit R, # <u>19</u> Exhibit S, # <u>20</u> Exhibit T, # <u>21</u> Exhibit U, # <u>22</u> Exhibit V, # <u>23</u> Exhibit W, # <u>24</u> Exhibit X, # <u>25</u> Exhibit Y, # <u>26</u> Exhibit Z, # <u>27</u> Exhibit AA, # <u>28</u> Exhibit BB, # <u>29</u> Exhibit CC, # <u>30</u> Exhibit DD, # <u>31</u> Exhibit EE, # <u>32</u> Exhibit FF, # <u>33</u> Exhibit GG, # <u>34</u> Exhibit HH, # <u>35</u> Exhibit II, # <u>36</u> Exhibit JJ, # <u>37</u> Exhibit KK)(Mayo, Andrew) (Entered: 03/30/2018)
03/30/2018	<u>112</u>	[SEALED] DECLARATION of William R. Strohl, Ph.D. in Support of Genentech, Inc.'s Opposition to Baxalta's Motion for a Preliminary Injunction by Genentech, Inc.. (Attachments: # <u>1</u> Exhibit A, # <u>2</u> Exhibit B, # <u>3</u> Exhibit C, # <u>4</u> Exhibit D, # <u>5</u> Exhibit E, # <u>6</u> Exhibit F, # <u>7</u> Exhibit G, # <u>8</u> Exhibit H, # <u>9</u> Exhibit I, # <u>10</u> Exhibit J, # <u>11</u> Exhibit K, # <u>12</u> Exhibit L, # <u>13</u> Exhibit M, # <u>14</u> Exhibit N, # <u>15</u> Exhibit O, # <u>16</u> Exhibit P, # <u>17</u> Exhibit Q, # <u>18</u> Exhibit R, # <u>19</u> Exhibit S, # <u>20</u> Exhibit T, # <u>21</u> Exhibit U, # <u>22</u> Exhibit V, # <u>23</u> Exhibit W, # <u>24</u> Exhibit X, # <u>25</u> Exhibit Y, # <u>26</u> Exhibit Z, # <u>27</u> Exhibit AA, # <u>28</u> Exhibit BB, # <u>29</u> Exhibit CC, # <u>30</u> Exhibit DD, # <u>31</u> Exhibit EE, # <u>32</u> Exhibit FF, # <u>33</u> Exhibit GG, # <u>34</u> Exhibit HH, # <u>35</u> Exhibit II, # <u>36</u> Exhibit JJ, # <u>37</u> Exhibit KK, # <u>38</u> Exhibit LL, # <u>39</u> Exhibit MM, # <u>40</u> Exhibit NN, # <u>41</u> Exhibit OO, # <u>42</u> Exhibit PP, # <u>43</u> Exhibit QQ, # <u>44</u> Exhibit RR, # <u>45</u> Exhibit SS, # <u>46</u> Exhibit TT, # <u>47</u> Exhibit UU, # <u>48</u> Exhibit VV, # <u>49</u> Exhibit WW, # <u>50</u> Exhibit XX, # <u>51</u> Exhibit YY, # <u>52</u> Exhibit ZZ, # <u>53</u> Exhibit AAA, # <u>54</u> Exhibit BBB, # <u>55</u> Exhibit CCC, # <u>56</u> Exhibit DDD, # <u>57</u> Exhibit EEE, # <u>58</u> Exhibit FFF, # <u>59</u> Exhibit GGG, # <u>60</u> Exhibit HHH, # <u>61</u> Exhibit III, # <u>62</u> Exhibit JJJ, # <u>63</u> Exhibit KKK, # <u>64</u> Exhibit LLL, # <u>65</u> Exhibit MMM, # <u>66</u> Exhibit NNN, # <u>67</u> Exhibit OOO, # <u>68</u> Exhibit PPP, # <u>69</u> Exhibit QQQ, # <u>70</u> Exhibit RRR, # <u>71</u> Exhibit SSS, # <u>72</u> Exhibit TTT, # <u>73</u> Exhibit UUU, # <u>74</u> Exhibit VVV, # <u>75</u> Exhibit WWW, # <u>76</u> Exhibit XXX, # <u>77</u> Exhibit YYY, # <u>78</u> Exhibit ZZZ, # <u>79</u> Exhibit AAAA, # <u>80</u> Exhibit BBBB)(Mayo, Andrew) (Entered: 03/30/2018)
04/03/2018	<u>113</u>	NOTICE OF SERVICE of Chugai Pharmaceutical Co., Ltd.'s First Amended Preliminary Identification of Claim Terms that Need to be Construed filed by Chugai Pharmaceutical Co., Ltd..(Keller, Karen) (Entered: 04/03/2018)



04/04/2018	<u>114</u>	NOTICE to Take Deposition of Gallia Levy on April 5, 2018 filed by Baxalta GmbH, Baxalta Incorporated.(Dudash, Amy) (Entered: 04/04/2018)
04/04/2018	<u>115</u>	NOTICE to Take Deposition of Gina Chapman on April 6, 2018 filed by Baxalta GmbH, Baxalta Incorporated.(Dudash, Amy) (Entered: 04/04/2018)
04/06/2018	<u>116</u>	REDACTED VERSION of <u>108</u> Declaration ( <i>of Jerry A. Hausman, Ph.D.</i> ) by Genentech, Inc.. (Mayo, Andrew) (Entered: 04/06/2018)
04/06/2018	<u>117</u>	REDACTED VERSION of <u>109</u> Declaration ( <i>of Michael U. Callaghan, M.D.</i> ) by Genentech, Inc.. (Mayo, Andrew) (Entered: 04/06/2018)
04/06/2018	<u>118</u>	REDACTED VERSION of <u>110</u> Declaration ( <i>of Guy A. Young, M.D.</i> ) by Genentech, Inc.. (Mayo, Andrew) (Entered: 04/06/2018)
04/06/2018	<u>119</u>	REDACTED VERSION of <u>111</u> Declaration,,, ( <i>of John P. Sheehan, M.D.</i> ) by Genentech, Inc.. (Attachments: # <u>1</u> Exhibit A–Z, # <u>2</u> Exhibit AA–KK)(Mayo, Andrew) (Entered: 04/06/2018)
04/06/2018	<u>120</u>	REDACTED VERSION of <u>112</u> Declaration,,,,, ( <i>of William R. Strohl, Ph.D.</i> ) by Genentech, Inc.. (Attachments: # <u>1</u> Exhibit A–K, # <u>2</u> Exhibit L–Z, # <u>3</u> Exhibit AA–LL, # <u>4</u> Exhibit MM–GGG, # <u>5</u> Exhibit HHH–SSS, # <u>6</u> Exhibit TTT–BBBB)(Mayo, Andrew) (Entered: 04/06/2018)
04/06/2018	<u>121</u>	NOTICE to Take Deposition of Gallia Levy (REVISED) on April 5, 2018 filed by Baxalta GmbH, Baxalta Incorporated.(Dudash, Amy) (Entered: 04/06/2018)
04/06/2018	<u>122</u>	NOTICE to Take Deposition of Gina Chapman (REVISED) on April 6, 2018 filed by Baxalta GmbH, Baxalta Incorporated.(Dudash, Amy) (Entered: 04/06/2018)
04/06/2018	<u>123</u>	CLAIM Construction Chart by Baxalta GmbH, Baxalta Incorporated. (Dudash, Amy) (Entered: 04/06/2018)
04/11/2018	<u>124</u>	DECLARATION – <i>Rebuttal of Juan Carlos Almagro, Ph.D., in Support of Plaintiffs' Reply Brief for Preliminary Injunction</i> by Baxalta GmbH, Baxalta Incorporated. (Attachments: # <u>1</u> Exhibit 1, # <u>2</u> Exhibit 2, # <u>3</u> Exhibit 3, # <u>4</u> Exhibit 4, # <u>5</u> Exhibit 8, # <u>6</u> Exhibit 9, # <u>7</u> Exhibit 10, # <u>8</u> Exhibit 11, # <u>9</u> Exhibit 12, # <u>10</u> Exhibit 13, # <u>11</u> Exhibit 14, # <u>12</u> Exhibit 15, # <u>13</u> Exhibit 16, # <u>14</u> Exhibit 17, # <u>15</u> Exhibit 18, # <u>16</u> Exhibit 19, # <u>17</u> Exhibit 20, # <u>18</u> Exhibit 21, # <u>19</u> Exhibit 22, # <u>20</u> Exhibit 23, # <u>21</u> Exhibit 24, # <u>22</u> Exhibit 25)(Dudash, Amy) (Entered: 04/11/2018)
04/11/2018	<u>125</u>	[SEALED] EXHIBIT re <u>124</u> Declaration,, <i>Exhibits 5, 6, and 7 to Rebuttal Declaration of Juan Carlos Almagro, PhD., in Support of Plaintiffs' Reply Brief for Preliminary Injunction</i> by Baxalta GmbH, Baxalta Incorporated. (Dudash, Amy) (Entered: 04/11/2018)
04/11/2018	<u>126</u>	[SEALED] DECLARATION by Baxalta GmbH, Baxalta Incorporated. (Attachments: # <u>1</u> Exhibit 1, # <u>2</u> Exhibit 2, # <u>3</u> Exhibit 3, # <u>4</u> Exhibit 4, # <u>5</u> Exhibit 5, # <u>6</u> Exhibit 6, # <u>7</u> Exhibit 7, # <u>8</u> Exhibit 8, # <u>9</u> Exhibit 9, # <u>10</u> Exhibit 10, # <u>11</u> Exhibit 11, # <u>12</u> Exhibit 12, # <u>13</u> Exhibit 13, # <u>14</u> Exhibit 14, # <u>15</u> Exhibit 15, # <u>16</u> Exhibit 16, # <u>17</u> Exhibit 17, # <u>18</u> Exhibit 18, # <u>19</u> Exhibit 19, # <u>20</u> Exhibit 20, # <u>21</u> Exhibit 21, # <u>22</u> Exhibit 22, # <u>23</u> Exhibit 23, # <u>24</u> Exhibit 24, # <u>25</u> Exhibit 27, # <u>26</u> Exhibit 28, # <u>27</u> Exhibit 29, # <u>28</u> Exhibit 30, # <u>29</u> Exhibit 31, # <u>30</u> Exhibit 32, # <u>31</u> Exhibit 33, # <u>32</u> Exhibit 34, # <u>33</u> Exhibit 35, # <u>34</u> Exhibit 36, # <u>35</u> Exhibit 37, # <u>36</u> Exhibit 38, # <u>37</u> Exhibit 39, # <u>38</u> Exhibit 40)(Dudash, Amy) Modified on 4/12/2018 (etg). (Entered: 04/11/2018)
04/11/2018	<u>127</u>	[SEALED] EXHIBIT re <u>126</u> Declaration,,, <i>Exhibits 25 and 26 to Rebuttal Declaration of Anthony A. Kossiakoff, Ph.D., in Support of Plaintiffs' Reply Brief for Preliminary Injunction</i> by Baxalta GmbH, Baxalta Incorporated. (Dudash, Amy) (Entered: 04/11/2018)
04/11/2018	<u>128</u>	[SEALED] DECLARATION of <i>Sriram Krishnaswamy in Support of Plaintiff's Reply Brief for Preliminary Injunction</i> by Baxalta GmbH, Baxalta Incorporated. (Attachments: # <u>1</u> Exhibit 5, # <u>2</u> Exhibit 7)(Dudash, Amy) (Entered: 04/11/2018)
04/11/2018	<u>129</u>	EXHIBIT re <u>128</u> Declaration <i>Exhibit 1</i> by Baxalta GmbH, Baxalta Incorporated. (Attachments: # <u>1</u> Exhibit 2, # <u>2</u> Exhibit 3, # <u>3</u> Exhibit 4, # <u>4</u> Exhibit 6, # <u>5</u> Exhibit 8)(Dudash, Amy) (Entered: 04/11/2018)
04/12/2018		CORRECTING ENTRY: As per the request of counsel, D.I. 126 has been placed under seal. (etg) (Entered: 04/12/2018)

04/12/2018	<u>130</u>	[SEALED] Amended EXHIBIT re <u>126</u> Declaration,,, <u>127</u> Exhibit to a Document, <i>Exhibit 26 to Rebuttal Declaration of Anthony A. Kossiakoff, PhD, in Support of Plaintiffs' Reply Brief for Preliminary Injunction</i> by Baxalta GmbH, Baxalta Incorporated. (Dudash, Amy) (Entered: 04/12/2018)
04/16/2018	<u>131</u>	AMENDED DOCUMENT by Baxalta GmbH, Baxalta Incorporated. Amendment to <u>123</u> Claim Construction Chart . (Dudash, Amy) (Entered: 04/16/2018)
04/16/2018	<u>132</u>	NOTICE to Take Deposition of Guy Young on April 25, 2018 filed by Baxalta GmbH, Baxalta Incorporated.(Dudash, Amy) (Entered: 04/16/2018)
04/16/2018	<u>133</u>	NOTICE to Take Deposition of Jerry Hausman on April 18, 2018 filed by Baxalta GmbH, Baxalta Incorporated.(Dudash, Amy) (Entered: 04/16/2018)
04/16/2018	<u>134</u>	NOTICE to Take Deposition of Michael Callaghan on April 20, 2018 filed by Baxalta GmbH, Baxalta Incorporated.(Dudash, Amy) (Entered: 04/16/2018)
04/16/2018	<u>135</u>	NOTICE to Take Deposition of William Strohl on April 18, 2018 filed by Baxalta GmbH, Baxalta Incorporated.(Dudash, Amy) (Entered: 04/16/2018)
04/16/2018	<u>136</u>	NOTICE to Take Deposition of John Sheehan on April 23, 2018 filed by Baxalta GmbH, Baxalta Incorporated.(Dudash, Amy) (Entered: 04/16/2018)
04/17/2018	<u>137</u>	MOTION for Pro Hac Vice Appearance of Attorney Jason C. White – filed by Baxalta GmbH, Baxalta Incorporated. (Dudash, Amy) (Entered: 04/17/2018)
04/18/2018	<u>138</u>	SEALED DECLARATION re <u>126</u> Declaration,,, <i>of Anthony Kossiakoff</i> by Baxalta GmbH, Baxalta Incorporated. (Attachments: # <u>1</u> Exhibit 25 – REDACTED, # <u>2</u> Exhibit 26 – REDACTED)(Dudash, Amy) (Entered: 04/18/2018)
04/18/2018	<u>139</u>	DECLARATION re <u>128</u> Declaration <i>of Sriram Krishnaswamy</i> – REDACTED by Baxalta GmbH, Baxalta Incorporated. (Attachments: # <u>1</u> Exhibit 5 – REDACTED, # <u>2</u> Exhibit 7 – REDACTED)(Dudash, Amy) (Entered: 04/18/2018)
04/19/2018		SO ORDERED, re <u>137</u> MOTION for Pro Hac Vice Appearance of Attorney Jason C. White filed by Baxalta GmbH, Baxalta Incorporated. Signed by Judge Leonard P. Stark on 4/19/18. (ntl) (Entered: 04/19/2018)
04/23/2018	<u>140</u>	NOTICE OF SERVICE of (1) Defendant Chugai Pharmaceutical Co., Ltd.'s Responses and Objections to Plaintiffs First Set of Requests for Admission (Nos. 1–3) filed by Chugai Pharmaceutical Co., Ltd..(Keller, Karen) (Entered: 04/23/2018)
04/23/2018	<u>141</u>	NOTICE OF SERVICE of Genentech's Objections and Responses to Baxalta's First Set of Requests for Admission filed by Genentech, Inc..(Mayo, Andrew) (Entered: 04/23/2018)
04/24/2018	<u>142</u>	NOTICE to Take Deposition of Juan Carlos Almagro, Ph.D. on April 26, 2018 filed by Genentech, Inc..(Mayo, Andrew) (Entered: 04/24/2018)
04/26/2018		Pro Hac Vice Attorney Jason C. White for Baxalta GmbH, Baxalta Incorporated added for electronic noticing. Pursuant to Local Rule 83.5 (d)., Delaware counsel shall be the registered users of CM/ECF and shall be required to file all papers. (crb) (Entered: 04/26/2018)
04/26/2018	<u>143</u>	NOTICE OF SERVICE of Defendant Genentech, Inc.'s Amended Initial Disclosures Pursuant to Section 3 of the Default Standard for Discovery filed by Genentech, Inc..(Balick, Steven) (Entered: 04/26/2018)
04/30/2018	<u>144</u>	NOTICE to Take Deposition of W. Christopher Bakewell on May 2, 2018 filed by Genentech, Inc..(Mayo, Andrew) (Entered: 04/30/2018)
04/30/2018	<u>145</u>	NOTICE to Take Deposition of Sriram Krishnaswamy, Ph.D. on May 3, 2018 filed by Genentech, Inc..(Mayo, Andrew) (Entered: 04/30/2018)
04/30/2018	<u>146</u>	NOTICE to Take Deposition of Louis M. Aledort, M.D. on May 4, 2018 filed by Genentech, Inc..(Mayo, Andrew) (Entered: 04/30/2018)
05/02/2018	<u>147</u>	STIPULATION Seeking to Revise the Markman Briefing Schedule, by Genentech, Inc.. (Balick, Steven) (Entered: 05/02/2018)
05/04/2018		Case Reassigned to Judge Timothy Belcher Dyk of the U.S. Court of Appeals for Federal Circuit. Please include the initials of the Judge (TBD) after the case number on all documents

		filed. (rjb) (Entered: 05/04/2018)
05/08/2018	<u>148</u>	NOTICE to Take Deposition of Anthony A. Kossiakoff, Ph.D. on May 10, 2018 filed by Genentech, Inc..(Mayo, Andrew) (Entered: 05/08/2018)
05/09/2018		ORAL ORDER: Status conference to be held at 2:00 p.m., Monday, May 14, 2018, in Courtroom 203 of the U.S. Court of Appeals for the Federal Circuit, Washington, DC. The parties should be prepared to discuss the following at the status conference: resetting the date for the Markman hearing and for trial and what if any claim–construction issues will need to be addressed as part of the preliminary–injunction proceedings. The deadlines for Markman briefing are extended as stipulated at ECF No. <u>147</u> to May 18, 2018, for the opening briefs and to July 2, 2018, for the responsive briefs. Ordered by Judge Timothy B. Dyk on 5/9/2018. (nmg) (Entered: 05/09/2018)
05/09/2018	<u>149</u>	STIPULATION Requesting Additional Pages for Preliminary Injunction Answering and Reply Briefs, by Genentech, Inc.. (Balick, Steven) (Entered: 05/09/2018)
05/11/2018	<u>150</u>	ORDER: Going forward, the parties shall provide two copies of all filings made on the docket in this action to the following address:Chambers of Hon. Timothy B. Dyk, United States Court of Appeals for the Federal Circuit, 717 Madison Place N.W., Washington, DC 20439. Signed by Judge Timothy Belcher Dyk on 5/11/2018. (fms) (Entered: 05/11/2018)
05/11/2018	<u>151</u>	ORDER approving <u>149</u> Stipulation Requesting Additional Pages for Preliminary Injunction Answering and Reply Briefs. Signed by Judge Timothy Belcher Dyk on 5/11/2018. (fms) (Entered: 05/11/2018)
05/14/2018		Minute Entry for proceedings held before Judge Timothy Belcher Dyk – Status Conference held on 5/14/2018. The Markman Hearing set for 8/2/2018 is rescheduled for 9/13/2018 at 10:00 AM at the U.S. Court of Appeals for the Federal Circuit, Washington, DC. The parties will submit additional proposed revisions to the schedule by Friday, May 18, 2018. (Court Reporter present.) (crb) (Entered: 05/14/2018)
05/15/2018	<u>152</u>	MOTION for Pro Hac Vice Appearance of Attorney Julie S. Goldemberg – filed by Baxalta GmbH, Baxalta Incorporated. (Dudash, Amy) (Entered: 05/15/2018)
05/15/2018	<u>153</u>	[SEALED] DECLARATION of David E. Cole in Support of Genentech's Memorandum of Law in Opposition to Plaintiff's Motion for a Preliminary Injunction, by Genentech, Inc.. (Attachments: # <u>1</u> Exhibit 1–25)(Balick, Steven) (Entered: 05/15/2018)
05/15/2018	<u>154</u>	[SEALED] ANSWERING BRIEF in Opposition re <u>41</u> MOTION for Preliminary Injunction , filed by Genentech, Inc..Reply Brief due date per Local Rules is 5/22/2018. (Balick, Steven) (Entered: 05/15/2018)
05/16/2018	<u>155</u>	Official Transcript of Status Conference held on 5/14/2018 before Judge Dyk. Court Reporter/Transcriber Keith A. Wilkerson, 1–800–211–3376. Transcript may be viewed at the court public terminal or purchased through the Court Reporter/Transcriber before the deadline for Release of Transcript Restriction. After that date it may be obtained through PACER Redaction Request due 6/6/2018. Redacted Transcript Deadline set for 6/18/2018. Release of Transcript Restriction set for 8/14/2018. (nmf) (Entered: 05/17/2018)
05/18/2018	<u>156</u>	ORDER granting <u>152</u> MOTION for Pro Hac Vice Appearance of Attorney Julie S. Goldemberg filed by Baxalta GmbH, Baxalta Incorporated. Signed by Judge Timothy Belcher Dyk on 5/18/2018. (fms) (Entered: 05/18/2018)
05/18/2018	<u>157</u>	STIPULATION Seeking to Modify Scheduling Order, by Genentech, Inc.. (Balick, Steven) (Entered: 05/18/2018)
05/18/2018	<u>158</u>	CLAIM CONSTRUCTION OPENING BRIEF filed by Baxalta GmbH, Baxalta Incorporated. (Dudash, Amy) (Entered: 05/18/2018)
05/18/2018	<u>159</u>	DECLARATION re <u>158</u> Claim Construction Opening Brief of Amy Dudash by Baxalta GmbH, Baxalta Incorporated. (Attachments: # <u>1</u> Exhibit A, # <u>2</u> Exhibit B, # <u>3</u> Exhibit C, # <u>4</u> Exhibit D, # <u>5</u> Exhibit E, # <u>6</u> Exhibit F, # <u>7</u> Exhibit G, # <u>8</u> Exhibit H, # <u>9</u> Exhibit I)(Dudash, Amy) (Entered: 05/18/2018)
05/18/2018	<u>160</u>	[SEALED] CLAIM CONSTRUCTION OPENING BRIEF filed by Genentech, Inc.. (Balick, Steven) (Entered: 05/18/2018)

05/18/2018	<u>161</u>	[SEALED] DECLARATION re <u>160</u> Claim Construction Opening Brief ( <i>Declaration of William R. Strohl, Ph.D.</i> ) by Genentech, Inc.. (Attachments: # <u>1</u> Exhibit A–J)(Balick, Steven) (Entered: 05/18/2018)
05/18/2018	<u>162</u>	[SEALED] DECLARATION re <u>160</u> Claim Construction Opening Brief ( <i>Declaration of David E. Cole</i> ) by Genentech, Inc.. (Attachments: # <u>1</u> Exhibit 1–3)(Balick, Steven) (Entered: 05/18/2018)
05/18/2018	<u>163</u>	[SEALED] DECLARATION re <u>160</u> Claim Construction Opening Brief ( <i>Declaration of John P. Sheehan, M.D.</i> ) by Genentech, Inc.. (Attachments: # <u>1</u> Exhibit A–H)(Balick, Steven) (Entered: 05/18/2018)
05/18/2018	<u>164</u>	JOINDER by Chugai Pharmaceutical Co., Ltd., joining in <u>160</u> Claim Construction Opening Brief . (Keller, Karen) (Entered: 05/18/2018)
05/21/2018	<u>165</u>	SCHEDULING ORDER: The parties shall file any comments by joint letter, not to exceed five double–spaced pages, by May 21, 2018, by 6:00 p.m. ( Joinder of Parties due by 6/7/2018., Amended Pleadings due by 6/7/2018., Fact Discovery completed by 10/5/2018., Opening Expert Reports due by 11/9/2018., Rebuttal Expert Reports due by 12/14/2018., Expert Discovery due by 2/15/2019., Reply expert reports shall be served on or before January 11, 2019. A two–day Motion Hearing on the motion for a preliminary injunction is set for 6/13/2018 at 09:30 AM in Courtroom 4A before Judge Timothy Belcher Dyk of the U.S. District Court for the District of Delaware, Wilmington, DE., AN Oral Argument on the motion to dismiss is set for 6/12/2018 at 03:30 PM before Judge Timothy Belcher Dyk at the U.S. District Court for the District of Delaware, Wilmington, DE., Claim Construction Answering Brief due by 7/2/2018., A half–day Markman Hearing is set for 9/13/2018 at 10:00 AM before Judge Timothy Belcher Dyk at the U.S. Court of Appeals for the Federal Circuit, Washington, DC.), Set Briefing Schedule: re <u>41</u> MOTION for Preliminary Injunction (Reply Brief due 5/25/2018.). Any party seeking leave to file a dispositive motion shall file a letter brief, not to exceed five double–spaced pages, by March 1, 2019. Date for trial to be scheduled at a later date. (See order for further details and deadlines). Signed by Judge Timothy Belcher Dyk on 5/18/2018. (fms) (Entered: 05/21/2018)
05/21/2018	<u>166</u>	AMENDED DOCUMENT by Baxalta GmbH, Baxalta Incorporated. – <i>Second Amended Joint Claim Construction Chart for '590 Patent</i> . (Dudash, Amy) (Entered: 05/21/2018)
05/21/2018	<u>167</u>	Letter to Honorable Timothy B. Dyk from Baxalta Incorporated, Baxalta GmbH and Chugai Pharmaceutical Co., Ltd. regarding May 18, 2018 Proposed Order Modifying Schedule – re <u>165</u> Scheduling Order,,,,,, Set Briefing Schedule,,,,,. (Dudash, Amy) (Entered: 05/21/2018)
05/22/2018	<u>168</u>	REDACTED VERSION of <u>153</u> Declaration of <i>David E. Cole</i> by Genentech, Inc.. (Attachments: # <u>1</u> Exhibit 1–25)(Mayo, Andrew) (Entered: 05/22/2018)
05/22/2018	<u>169</u>	REDACTED VERSION of <u>154</u> Answering Brief in Opposition by Genentech, Inc.. (Mayo, Andrew) (Entered: 05/22/2018)
05/22/2018	<u>170</u>	MOTION for Pro Hac Vice Appearance of Attorney Karon N. Fowler – filed by Baxalta GmbH, Baxalta Incorporated. (Dudash, Amy) (Entered: 05/22/2018)
05/22/2018		ORAL ORDER: A teleconference has been set in this matter for Wednesday, May 23, 2018, at 4:00 PM. Counsel for plaintiffs shall initiate the call to chambers once all participants are on the line. Ordered by Judge Timothy B. Dyk on 5/22/2018. (Entered: 05/22/2018)
05/22/2018	<u>171</u>	ORDER granting <u>170</u> MOTION for Pro Hac Vice Appearance of Attorney Karon N. Fowler filed by Baxalta GmbH, Baxalta Incorporated. Signed by Judge Timothy Belcher Dyk on 5/22/2018. (fms) (Entered: 05/22/2018)
05/22/2018	<u>172</u>	NOTICE of Supplemental Developments Regarding Baxalta's Motion for a Preliminary Injunction, by Genentech, Inc. re <u>154</u> Answering Brief in Opposition (Balick, Steven) (Entered: 05/22/2018)
05/22/2018	<u>173</u>	DECLARATION re <u>154</u> Answering Brief in Opposition ( <i>Supplemental Declaration of David E. Cole in Support of Genentech's Opposition to Plaintiffs' Motion for a Preliminary Injunction</i> ), by Genentech, Inc.. (Attachments: # <u>1</u> Exhibit 26–28)(Balick, Steven) (Entered: 05/22/2018)
05/23/2018		Pro Hac Vice Attorney Karon N. Fowler for Baxalta GmbH, Baxalta Incorporated added for electronic noticing. Pursuant to Local Rule 83.5 (d)., Delaware counsel shall be the registered users of CM/ECF and shall be required to file all papers. (crb) (Entered: 05/23/2018)

05/23/2018		Minute Entry for proceedings held before Judge Timothy Belcher Dyk – Telephone Conference held on 5/23/2018. (Court Reporter present.) (sar) (Entered: 05/24/2018)
05/23/2018	<u>174</u>	ORDER MODIFYING SCHEDULE : All jurisdictional discovery for purposes of Defendant Chugai's pending motion to dismiss shall be completed by May 23, 2018. Set Briefing Schedule: re <u>41</u> MOTION for Preliminary Injunction (Reply Brief due 5/25/2018.) Joinder of Parties due by 6/7/2018. Amended Pleadings due by 6/7/2018. Fact Discovery completed by 10/5/2018. Expert Discovery due by 2/15/2019. A Motion Hearing is set for 6/13/2018 at 09:30 AM in Courtroom 4A at the U.S. District Court for the District of Delaware, Wilmington, DE before Judge Timothy Belcher Dyk An Oral Argument is set for 6/12/2018 at 03:30 PM in Courtroom 4A at the U.S. District Court for the District of Delaware, Wilmington, DE before Judge Timothy Belcher Dyk. Claim Construction Answering Brief due by 7/2/2018. A Markman Hearing is set for 9/13/2018 at 10:00 AM before Judge Timothy Belcher Dyk at the U.S. Court of Appeals for the Federal Circuit, Washington DC. Any party seeking leave to file a dispositive motion shall file a letter brief, not to exceed five double-spaced pages, by March 1, 2019. Date for trial to be scheduled at a later date. (See order for further details and deadlines). Signed by Judge Timothy Belcher Dyk on 5/23/18. (sar) (Entered: 05/24/2018)
05/24/2018	<u>175</u>	NOTICE OF SERVICE of Defendant Chugai Pharmaceutical Co., Ltd.'s Supplemental Responses to Plaintiffs' First Set of Interrogatories (Nos. 6–7) filed by Chugai Pharmaceutical Co., Ltd..(Keller, Karen) (Entered: 05/24/2018)
05/25/2018	<u>176</u>	REDACTED VERSION of <u>160</u> Claim Construction Opening Brief by Genentech, Inc.. (Mayo, Andrew) (Entered: 05/25/2018)
05/25/2018	<u>177</u>	REDACTED VERSION of <u>161</u> Declaration of William R. Strohl, Ph.D. by Genentech, Inc.. (Attachments: # <u>1</u> Exhibit A–J)(Mayo, Andrew) (Entered: 05/25/2018)
05/25/2018	<u>178</u>	REDACTED VERSION of <u>162</u> Declaration of David E. Cole by Genentech, Inc.. (Attachments: # <u>1</u> Exhibit 1–3)(Mayo, Andrew) (Entered: 05/25/2018)
05/25/2018	<u>179</u>	REDACTED VERSION of <u>163</u> Declaration of John P. Sheehan, M.D. by Genentech, Inc.. (Attachments: # <u>1</u> Exhibit A–H)(Mayo, Andrew) (Entered: 05/25/2018)
05/25/2018	<u>180</u>	[SEALED] REPLY to Response to Motion re <u>41</u> MOTION for Preliminary Injunction filed by Baxalta GmbH, Baxalta Incorporated. (Attachments: # <u>1</u> Affidavit Amy Dudash, # <u>2</u> Exhibit A, # <u>3</u> Exhibit B, # <u>4</u> Exhibit C, # <u>5</u> Exhibit D, # <u>6</u> Exhibit E, # <u>7</u> Exhibit F, # <u>8</u> Exhibit G, # <u>9</u> Exhibit H, # <u>10</u> Exhibit I, # <u>11</u> Exhibit J, # <u>12</u> Exhibit K, # <u>13</u> Exhibit L, # <u>14</u> Exhibit M, # <u>15</u> Exhibit N, # <u>16</u> Exhibit O, # <u>17</u> Exhibit P, # <u>18</u> Exhibit Q, # <u>19</u> Exhibit R, # <u>20</u> Exhibit S, # <u>21</u> Exhibit T, # <u>22</u> Exhibit U, # <u>23</u> Exhibit V, # <u>24</u> Exhibit W, # <u>25</u> Exhibit X, # <u>26</u> Exhibit Y, # <u>27</u> Exhibit Z, # <u>28</u> Exhibit AA, # <u>29</u> Exhibit BB, # <u>30</u> Exhibit CC, # <u>31</u> Exhibit DD, # <u>32</u> Exhibit EE, # <u>33</u> Exhibit FF, # <u>34</u> Exhibit GG, # <u>35</u> Exhibit HH, # <u>36</u> Exhibit II, # <u>37</u> Exhibit JJ)(Dudash, Amy) (Entered: 05/25/2018)
05/25/2018	<u>181</u>	CERTIFICATE OF SERVICE of Baxalta's Sealed Reply in Support of Motion for Preliminary Injunction by Baxalta GmbH, Baxalta Incorporated re <u>180</u> Reply to Response to Motion,,, (Dudash, Amy) (Entered: 05/25/2018)
05/29/2018	<u>182</u>	MOTION for Leave to permit Patients for Access to Advanced Hemophilia Therapy to File an Amicus Brief – filed by Patients for Access to Advanced Hemophilia Therapy. (Attachments: # <u>1</u> Proposed Amicus Curiae Brief in Opposition to Plaintiff's Motion for a Preliminary Injunction)(nmf) (Entered: 05/30/2018)
05/30/2018	<u>183</u>	ORDER re <u>182</u> MOTION for Leave to permit to File an Amicus Brief filed by Patients for Access to Advanced Hemophilia Therapy. No later than June 6, 2018, Plaintiffs shall file a letter, not to exceed one page, containing any objections to the filing of the proposed amicus brief. Signed by Judge Timothy Belcher Dyk on 5/30/2018. (fms) (Entered: 05/30/2018)
05/30/2018	<u>184</u>	Official Transcript of Telephone Conference held on 5/23/2018 before Judge Timothy Belcher Dyk. Court Reporter/Transcriber Cynthia J. Conforti, CSR, CRR, Telephone number 800.211.3376. Transcript may be viewed at the court public terminal or purchased through the Court Reporter/Transcriber before the deadline for Release of Transcript Restriction. After that date it may be obtained through PACER. Redaction Request due 6/20/2018. Redacted Transcript Deadline set for 7/2/2018. Release of Transcript Restriction set for 8/28/2018. (fms) (Entered: 05/30/2018)
05/30/2018	<u>185</u>	ORDER denying the request to conduct a Rule 30(b)(6) deposition of Chugai on jurisdictional matters. See order for further details. Signed by Judge Timothy Belcher Dyk on 5/30/2018.

		(nmf) (Entered: 05/31/2018)
06/01/2018	<u>186</u>	REDACTED VERSION of <u>180</u> Reply to Response to Motion,, by Baxalta GmbH, Baxalta Incorporated. (Attachments: # <u>1</u> Affidavit Amy Dudash, # <u>2</u> Exhibit A–JJ)(Dudash, Amy) (Entered: 06/01/2018)
06/05/2018	<u>187</u>	[SEALED] Letter to Honorable Timothy B. Dyk from Amy Dudash regarding Motion to Dismiss – re <u>18</u> MOTION to Dismiss for Lack of Jurisdiction Over the Person . (Attachments: # <u>1</u> Exhibit A, # <u>2</u> Exhibit B, # <u>3</u> Exhibit C, # <u>4</u> Exhibit D, # <u>5</u> Exhibit E, # <u>6</u> Exhibit F, # <u>7</u> Exhibit G, # <u>8</u> Exhibit H, # <u>9</u> Exhibit I, # <u>10</u> Exhibit J, # <u>11</u> Exhibit K, # <u>12</u> Exhibit L, # <u>13</u> Exhibit M, # <u>14</u> Exhibit N, # <u>15</u> Exhibit O, # <u>16</u> Exhibit P, # <u>17</u> Exhibit Q, # <u>18</u> Exhibit R, # <u>19</u> Exhibit S, # <u>20</u> Exhibit T)(Dudash, Amy) (Entered: 06/05/2018)
06/05/2018	<u>188</u>	CERTIFICATE OF SERVICE of Letter to The Honorable Timothy B. Dyk by Baxalta GmbH, Baxalta Incorporated re <u>187</u> Letter,, (Dudash, Amy) (Entered: 06/05/2018)
06/06/2018	<u>189</u>	Letter to The Honorable Timothy B. Dyk from Amy M. Dudash regarding plaintiff's submission of objections to Patents for Access to Advanced Hemophilia Therapy's ("PAAHT") Motion (D.I. 182) – re <u>183</u> Order, <u>182</u> MOTION for Leave to permit to File an Amicus Brief. (Dudash, Amy) (Entered: 06/06/2018)
06/06/2018		ORAL ORDER: The parties have raised a dispute concerning a privilege issue. No later than 5:00 PM on June 8, 2018, the parties shall file simultaneous letter briefs on this issue no longer than five double–spaced pages each. Ordered by Judge Timothy B. Dyk on 6/6/2018. (nmg) (Entered: 06/07/2018)
06/07/2018		ORAL ORDER: On May 29, 2018, Patients for Access to Advanced Hemophilia Therapy (PAAHT) moved for leave to file an amicus brief in opposition to plaintiffs motion for a preliminary injunction. ECF No. <u>182</u> . Plaintiffs responded with their objections to the filing of the amicus brief. ECF No. <u>189</u> . Having considered plaintiffs objections, PAAHTs motion is GRANTED. Ordered by Judge Timothy B. Dyk on 6/7/2018. (nmg) (Entered: 06/07/2018)
06/07/2018	<u>190</u>	Amicus Curiae Answering Brief in Opposition to D.I. <u>41</u> MOTION for Preliminary Injunction filed by Patients for Access to Advanced Hemophilia Therapy. Modified on 6/7/2018 (nmg). (Entered: 06/07/2018)
06/07/2018		NOTE: We have removed the deadline for a reply brief that was automatically generated by the system at ECF No. <u>190</u> . (nmg) (Entered: 06/07/2018)
06/07/2018	<u>191</u>	[SEALED] Amended EXHIBIT re <u>180</u> Reply to Response to Motion,,, <i>Exhibit B</i> by Baxalta GmbH, Baxalta Incorporated. (Dudash, Amy) (Entered: 06/07/2018)
06/07/2018	<u>192</u>	REDACTED VERSION of <u>191</u> Exhibit to a Document by Baxalta GmbH, Baxalta Incorporated. (Dudash, Amy) (Entered: 06/07/2018)
06/07/2018	<u>193</u>	[SEALED] MOTION to Amend/Correct <u>1</u> Complaint,, – filed by Baxalta GmbH, Baxalta Incorporated. (Attachments: # <u>1</u> Affidavit of Amy Dudash, # <u>2</u> Amended Complaint, # <u>3</u> Exhibit B, # <u>4</u> Exhibit C, # <u>5</u> Exhibit D)(Dudash, Amy) (Entered: 06/07/2018)
06/07/2018	<u>194</u>	CERTIFICATE OF SERVICE of Motion to Amend by Baxalta GmbH, Baxalta Incorporated re <u>193</u> MOTION to Amend/Correct <u>1</u> Complaint,, (Dudash, Amy) (Entered: 06/07/2018)
06/07/2018	<u>195</u>	[SEALED] MEMORANDUM in Support re <u>193</u> MOTION to Amend/Correct <u>1</u> Complaint,, filed by Baxalta GmbH, Baxalta Incorporated. Answering Brief/Response due date per Local Rules is 6/21/2018. (Dudash, Amy) (Entered: 06/07/2018)
06/08/2018	<u>196</u>	[SEALED] Letter to Honorable Timothy B. Dyk from Amy Dudash regarding Privilege Claim. (Attachments: # <u>1</u> Exhibit A)(Dudash, Amy) (Entered: 06/08/2018)
06/08/2018	<u>197</u>	CERTIFICATE OF SERVICE of Letter to The Honorable Timothy B. Dyk by Baxalta GmbH, Baxalta Incorporated re <u>196</u> Letter (Dudash, Amy) (Entered: 06/08/2018)
06/08/2018	<u>198</u>	[SEALED] Letter to The Honorable Timothy B. Dyk from Steven J. Balick regarding the Disputed Privilege Claim. (Attachments: # <u>1</u> Exhibit 1, # <u>2</u> Exhibit 2)(Balick, Steven) (Entered: 06/08/2018)
06/08/2018	<u>199</u>	NOTICE OF SERVICE of Plaintiffs' Supplemental Disclosure of Infringement Contentions filed by Baxalta GmbH, Baxalta Incorporated.(Dudash, Amy) (Entered: 06/08/2018)

06/08/2018	<u>200</u>	Letter to The Honorable Judge Timothy B. Dyk from Karen E. Keller – re <u>187</u> Letter,, (Keller, Karen) (Entered: 06/08/2018)
06/09/2018	<u>201</u>	[SEALED] Letter to The Honorable Timothy B. Dyk from Steven J. Balick regarding Claim Construction for Purposes of Baxalta's Motion for a Preliminary Injunction. (Balick, Steven) (Entered: 06/09/2018)
06/09/2018	<u>202</u>	Letter to Honorable Timothy B. Dyk from Amy M. Dudash regarding Supplemental Claim Construction Brief for Purposes of Preliminary Injunction. (Attachments: # <u>1</u> Exhibit 1 to Supplemental Claim Construction Letter Brief, # <u>2</u> Exhibit 2 to Supplemental Claim Construction Letter Brief)(Dudash, Amy) (Entered: 06/09/2018)
06/11/2018	<u>203</u>	REDACTED VERSION of <u>201</u> Letter by Genentech, Inc.. (Mayo, Andrew) (Entered: 06/11/2018)
06/12/2018	<u>204</u>	REDACTED VERSION of <u>187</u> Letter,, <i>to The Honorable Timothy B. Dyk from Amy M. Dudash</i> by Baxalta GmbH, Baxalta Incorporated. (Attachments: # <u>1</u> Redacted Exhibit A, # <u>2</u> Redacted Exhibit B, # <u>3</u> Redacted Exhibit C, # <u>4</u> Redacted Exhibit D, # <u>5</u> Redacted Exhibit E, # <u>6</u> Redacted Exhibit F, # <u>7</u> Redacted Exhibit G, # <u>8</u> Redacted Exhibit H, # <u>9</u> Redacted Exhibit I, # <u>10</u> Redacted Exhibit J, # <u>11</u> Redacted Exhibit K, # <u>12</u> Redacted Exhibit L, # <u>13</u> Redacted Exhibit M, # <u>14</u> Redacted Exhibit N, # <u>15</u> Redacted Exhibit O, # <u>16</u> Redacted Exhibit P, # <u>17</u> Redacted Exhibit Q, # <u>18</u> Redacted Exhibit R, # <u>19</u> Redacted Exhibit S, # <u>20</u> Redacted Exhibit T)(Dudash, Amy) (Entered: 06/12/2018)
06/12/2018		Minute Entry for proceedings held before Judge Timothy B. Dyk – Oral Argument held on 6/12/2018. Parties to provide additional supplemental briefing with respect to Exhibit JJ by close of business on 6/13/2018. (Court Reporter Susan Marie Migatz.)(fms) (Entered: 06/13/2018)
06/13/2018	<u>205</u>	[SEALED] Letter to The Honorable Timothy B. Dyk from Steven J. Balick regarding the Disputed Privilege Claim. (Balick, Steven) (Entered: 06/13/2018)
06/13/2018	<u>206</u>	[SEALED] Letter to Honorable Timothy B. Dyk from Amy Dudash regarding the Disputed Privilege Claim. (Attachments: # <u>1</u> Exhibit A)(Dudash, Amy) (Entered: 06/13/2018)
06/13/2018		Minute Entry for proceedings held before Judge Timothy Belcher Dyk – Motion Hearing held on 6/13/2018 re <u>41</u> Motion for Preliminary Injunction. (Court Reporter Susan Marie Migatz.)(ceg) (Entered: 06/14/2018)
06/14/2018	<u>207</u>	REDACTED VERSION of <u>193</u> MOTION to Amend/Correct <u>1</u> Complaint,, by Baxalta GmbH, Baxalta Incorporated. (Attachments: # <u>1</u> Affidavit of Amy Dudash, # <u>2</u> Amended Complaint, # <u>3</u> Exhibit B, # <u>4</u> Exhibit C, # <u>5</u> Exhibit D)(Dudash, Amy) (Entered: 06/14/2018)
06/14/2018	<u>208</u>	REDACTED VERSION of <u>195</u> MEMORANDUM in Support of <i>Motion to Amend/Correct Complaint</i> by Baxalta GmbH, Baxalta Incorporated. (Dudash, Amy) (Entered: 06/14/2018)
06/14/2018	<u>209</u>	REDACTED VERSION of <u>196</u> Letter to <i>Honorable Timothy B. Dyk from Amy Dudash regarding Privilege Claim</i> by Baxalta GmbH, Baxalta Incorporated. (Attachments: # <u>1</u> Exhibit A)(Dudash, Amy) (Entered: 06/14/2018)
06/14/2018		Minute Entry for proceedings held before Judge Timothy Belcher Dyk – Motion Hearing held on 6/14/2018 re <u>41</u> Motion for Preliminary Injunction. (Court Reporter Susan Marie Migatz.)(ceg) (Entered: 06/15/2018)
06/15/2018	<u>210</u>	REDACTED VERSION of <u>198</u> Letter by Genentech, Inc.. (Attachments: # <u>1</u> Exhibit 1–2)(Mayo, Andrew) (Entered: 06/15/2018)
06/19/2018		ORAL ORDER: Oral argument regarding the motion for a preliminary injunction has been set in this matter for Monday, July 2, 2018, at 1:30 PM at the U.S. Court of Appeals for the Federal Circuit, Washington, DC. The parties shall file proposed findings of fact and conclusions of law concerning the preliminary injunction, not to exceed 25 double–spaced pages, by the close of business on July 9, 2018. The parties shall simultaneously file reply proposed findings of fact and conclusions of law, not to exceed 10 double–spaced pages, by the close of business on July 16, 2018. Ordered by Judge Timothy B. Dyk on 6/19/2018. (nmf) (Entered: 06/19/2018)
06/19/2018	<u>211</u>	REDACTED VERSION of <u>205</u> Letter by Genentech, Inc.. (Balick, Steven) (Entered: 06/19/2018)
06/19/2018	<u>212</u>	REDACTED VERSION of <u>206</u> Letter by Baxalta GmbH, Baxalta Incorporated. (Attachments: # <u>1</u> Exhibit A – REDACTED)(Dudash, Amy) (Entered: 06/19/2018)

06/20/2018	<u>213</u>	Official Transcript of Motion to Dismiss held on 06-12-18 before Judge Timothy B. Dyk. Court Reporter/Transcriber Susan Migatz. Transcript may be viewed at the court public terminal or purchased through the Court Reporter/Transcriber before the deadline for Release of Transcript Restriction. After that date it may be obtained through PACER. Redaction Request due 7/11/2018. Redacted Transcript Deadline set for 7/23/2018. Release of Transcript Restriction set for 9/18/2018. (lad) (Entered: 06/20/2018)
06/20/2018	<u>214</u>	Official Transcript of Preliminary Injunction Hearing (Day 1) held on 06-13-18 before Judge Timothy B. Dyk. Court Reporter/Transcriber Susan Migatz. Transcript may be viewed at the court public terminal or purchased through the Court Reporter/Transcriber before the deadline for Release of Transcript Restriction. After that date it may be obtained through PACER. Redaction Request due 7/11/2018. Redacted Transcript Deadline set for 7/23/2018. Release of Transcript Restriction set for 9/18/2018. (lad) (Entered: 06/20/2018)
06/20/2018	<u>215</u>	Official Transcript of Preliminary Injunction Hearing (Day 2) held on 06-14-18 before Judge Timothy B. Dyk. Court Reporter/Transcriber Susan Migatz. Transcript may be viewed at the court public terminal or purchased through the Court Reporter/Transcriber before the deadline for Release of Transcript Restriction. After that date it may be obtained through PACER. Redaction Request due 7/11/2018. Redacted Transcript Deadline set for 7/23/2018. Release of Transcript Restriction set for 9/18/2018. (lad) (Entered: 06/20/2018)
06/21/2018	<u>216</u>	[SEALED] ANSWERING BRIEF in Opposition re <u>193</u> MOTION to Amend/Correct <u>1</u> Complaint,, filed by Genentech, Inc..Reply Brief due date per Local Rules is 6/28/2018. (Balick, Steven) (Entered: 06/21/2018)
06/21/2018	<u>217</u>	[SEALED] DECLARATION re <u>216</u> Answering Brief in Opposition ( <i>Declaration of Eric Alan Stone</i> ), by Genentech, Inc.. (Attachments: # <u>1</u> Exhibit A)(Balick, Steven) (Entered: 06/21/2018)
06/25/2018	<u>218</u>	AMENDED DOCUMENT by Baxalta GmbH, Baxalta Incorporated. Amendment to <u>42</u> MEMORANDUM in Support, <i>Exhibit A – Proposed Order</i> . (Dudash, Amy) (Entered: 06/25/2018)
06/26/2018	<u>219</u>	STIPULATION Regarding Extension of Deadlines for Submission of Opening and Responsive Proposed Findings of Fact and Conclusions of Law with Respect to the Pending Motion for Preliminary Injunction, and Regarding Deadline for Submission of Responsive Markman Briefs, by Genentech, Inc.. (Balick, Steven) (Entered: 06/26/2018)
06/26/2018	<u>220</u>	Joint STIPULATION re Chugai Pharmaceutical Co. Ltd. by Baxalta GmbH, Baxalta Incorporated. (Attachments: # <u>1</u> Exhibit A, # <u>2</u> Exhibit B, # <u>3</u> Exhibit C, # <u>4</u> Exhibit D)(Dudash, Amy) (Entered: 06/26/2018)
06/27/2018	<u>221</u>	AMENDED DOCUMENT by Baxalta GmbH, Baxalta Incorporated. Amendment to <u>220</u> Stipulation – <i>Corrected Exhibit B</i> . (Dudash, Amy) (Entered: 06/27/2018)
06/27/2018	<u>222</u>	ORDER approving <u>219</u> STIPULATION Regarding Extension of Deadlines for Submission of Opening and Responsive Proposed Findings of Fact and Conclusions of Law with Respect to the Pending Motion for Preliminary Injunction, and Regarding Deadline for Submission of Responsive Markman Briefs. The deadlines by which plaintiffs and defendant Genentech, Inc. shall file proposed findings of fact and conclusions of law with respect to the pending motion for preliminary injunction, as originally established in the Court's June 19, 2018 oral order, are extended, with simultaneous opening papers not to exceed 25 double-spaced pages due by 6:00 p.m. EDT on July 11, 2018, and simultaneous responsive papers not to exceed 10 double-spaced pages due by 6:00 p.m. EDT on July 18, 2018. ORDER Re-setting Scheduling Order deadlines: ( Claim Construction Answering Briefs due by 7/16/2018.) Signed by Judge Timothy Belcher Dyk on 6/26/2018. (nmf) (Entered: 06/27/2018)
06/28/2018	<u>223</u>	REDACTED VERSION of <u>216</u> Answering Brief in Opposition by Genentech, Inc.. (Mayo, Andrew) (Entered: 06/28/2018)
06/28/2018	<u>224</u>	REDACTED VERSION of <u>217</u> Declaration of <i>Eric Alan Stone</i> by Genentech, Inc.. (Mayo, Andrew) (Entered: 06/28/2018)
06/28/2018	<u>225</u>	[SEALED] REPLY to Response to Motion re <u>193</u> MOTION to Amend/Correct <u>1</u> Complaint,, filed by Baxalta GmbH, Baxalta Incorporated. (Dudash, Amy) (Entered: 06/28/2018)
06/28/2018	<u>226</u>	CERTIFICATE OF SERVICE of Baxalta's Sealed Reply Brief in Support of Motion to Amend the Complaint by Baxalta GmbH, Baxalta Incorporated re <u>225</u> Reply to Response to Motion (Dudash, Amy) (Entered: 06/28/2018)



07/02/2018		Minute Entry for proceedings held before Judge Timothy B. Dyk Oral argument held on 7/2/2018. Pursuant to the parties stipulation, Chugai shall make available a witness or witnesses for a Rule 30(b)(6) deposition. If the deposition is taken outside the United States, it shall be taken in accordance with the appropriate foreign law. Within five days of completion of the deposition, Baxalta shall file a proposed order dismissing Chugai in accordance with the stipulation. As stated on the record, Baxaltas motion for leave to amend its complaint is GRANTED. (Court Reporter Kenneth Norris present.) (nmg) (Entered: 07/02/2018)
07/05/2018	<u>227</u>	REDACTED VERSION of <u>225</u> Reply to Response to Motion by Baxalta GmbH, Baxalta Incorporated. (Dudash, Amy) (Entered: 07/05/2018)
07/09/2018	<u>228</u>	NOTICE of Withdrawal of Appearance of Jessica A. Stow as counsel for Plaintiffs by Baxalta GmbH, Baxalta Incorporated (Dudash, Amy) (Entered: 07/09/2018)
07/10/2018	<u>229</u>	Official Transcript of Oral Argument held on 7/2/2018 before Judge Timothy B. Dyk. Court Reporter/Transcriber Kenneth Norris, Telephone number 1-800-211-3376. Transcript may be viewed at the court public terminal or purchased through the Court Reporter/Transcriber before the deadline for Release of Transcript Restriction. After that date it may be obtained through PACER Redaction Request due 7/31/2018. Redacted Transcript Deadline set for 8/10/2018. Release of Transcript Restriction set for 10/9/2018. (crb) (Entered: 07/10/2018)
07/11/2018	<u>230</u>	[SEALED] Proposed Findings of Fact by Baxalta GmbH, Baxalta Incorporated. (Dudash, Amy) (Entered: 07/11/2018)
07/11/2018	<u>231</u>	CERTIFICATE OF SERVICE of Sealed Findings of Fact and Conclusions of Law by Baxalta GmbH, Baxalta Incorporated re <u>230</u> Proposed Findings of Fact (Dudash, Amy) (Entered: 07/11/2018)
07/11/2018	<u>232</u>	Proposed Findings of Fact by Genentech, Inc.. (Balick, Steven) (Entered: 07/11/2018)
07/16/2018	<u>233</u>	DECLARATION of David E. Cole in Support of Genentech's Responsive Claim Construction Brief Regarding Remaining Claim Terms, by Genentech, Inc.. (Attachments: # <u>1</u> Exhibit 1, # <u>2</u> Exhibit 2)(Balick, Steven) (Entered: 07/16/2018)
07/16/2018	<u>234</u>	CLAIM CONSTRUCTION ANSWERING BRIEF re <u>160</u> Claim Construction Opening Brief filed by Baxalta GmbH, Baxalta Incorporated. (Attachments: # <u>1</u> Affidavit of Amy Dudash, # <u>2</u> Exhibit 1)(Dudash, Amy) (Entered: 07/16/2018)
07/16/2018	<u>235</u>	[SEALED] CLAIM CONSTRUCTION ANSWERING BRIEF filed by Genentech, Inc.. (Balick, Steven) (Entered: 07/16/2018)
07/17/2018	<u>237</u>	JOINDER by Chugai Pharmaceutical Co., Ltd., joining in <u>235</u> Claim Construction Answering Brief . (Keller, Karen) (Entered: 07/17/2018)
07/17/2018	<u>238</u>	NOTICE OF SERVICE of Genentech, Inc.'s Third Set of Requests for the Production of Documents and Things to Baxalta Incorporated and Baxalta GmbH filed by Genentech, Inc..(Mayo, Andrew) (Entered: 07/17/2018)
07/18/2018	<u>239</u>	First AMENDED COMPLAINT for Patent Infringement and Declaratory Judgment of Patent Infringement against Chugai Pharmaceutical Co., Ltd., Genentech, Inc.- filed by Baxalta GmbH, Baxalta Incorporated.(Dudash, Amy) (Entered: 07/18/2018)
07/18/2018	<u>240</u>	ANSWER to Amended Complaint, re: <u>239</u> Amended Complaint with Jury Demand, COUNTERCLAIM against Baxalta GmbH, Baxalta Incorporated by Genentech, Inc.(nmf) (Entered: 07/18/2018)
07/18/2018		CORRECTING ENTRY: The answer which was previously docketed as a statement has been deleted and re-docketed as an answer to the amended complaint with counterclaim and jury demand now that the amended complaint has been filed. (SEE D.I. <u>240</u> ) (nmf) (Entered: 07/18/2018)
07/18/2018	<u>241</u>	REDACTED VERSION of <u>230</u> Proposed Findings of Fact by Baxalta GmbH, Baxalta Incorporated. (Dudash, Amy) (Entered: 07/18/2018)
07/18/2018	<u>242</u>	[SEALED] STATEMENT re <u>232</u> Proposed Findings of Fact by Baxalta GmbH, Baxalta Incorporated. (Dudash, Amy) (Entered: 07/18/2018)
07/18/2018	<u>243</u>	CERTIFICATE OF SERVICE of Baxalta's Sealed Response to Genentech's Findings of Fact and Conclusions of Law by Baxalta GmbH, Baxalta Incorporated re <u>242</u> Statement (Dudash, Amy) (Entered: 07/18/2018)

07/18/2018	<u>244</u>	STATEMENT [ <i>Defendant Genentech's Reply to Baxalta's Proposed Findings of Fact and Conclusions of Law (D.I. 230)</i> ] by Genentech, Inc.. (Mayo, Andrew) (Entered: 07/18/2018)
07/18/2018	<u>245</u>	[SEALED] Letter to The Honorable Timothy B. Dyk from Amy Dudash regarding Preliminary Injunction Exhibits. (Attachments: # <u>1</u> Exhibit A – Joint Exhibit List, # <u>2</u> Exhibit Vol. 1, # <u>3</u> Exhibit Vol. 2, # <u>4</u> Exhibit Vol. 3, # <u>5</u> Exhibit Vol. 4, # <u>6</u> Exhibit Vol. 5)(Dudash, Amy) (Entered: 07/18/2018)
07/18/2018	<u>246</u>	CERTIFICATE OF SERVICE of Letter to The Honorable Timothy B. Dyk by Baxalta GmbH, Baxalta Incorporated re <u>245</u> Letter, (Dudash, Amy) (Entered: 07/18/2018)
07/23/2018	<u>247</u>	Letter to The Honorable Timothy B. Dyk from Steven J. Balick regarding Genentech's Response to Baxalta's Objections to Preliminary Injunction Exhibits – re <u>245</u> Letter,. (Attachments: # <u>1</u> Exhibit A–D)(Balick, Steven) (Entered: 07/23/2018)
07/23/2018	<u>248</u>	ORDER Setting Follow–up Teleconference: Plaintiffs' Counsel to initiate the call. A Telephone Conference is set for 10/9/2018 at 10:30 AM Eastern Time before Judge Mary Pat Thyng to discuss ADR.Signed by Judge Mary Pat Thyng on 7/23/2018. (fms) (Entered: 07/23/2018)
07/23/2018	<u>249</u>	REDACTED VERSION of <u>235</u> Claim Construction Answering Brief by Genentech, Inc.. (Mayo, Andrew) (Entered: 07/23/2018)
07/23/2018	<u>250</u>	PROPOSED ORDER and Commission for Depositions to be Taken in Japan by Baxalta GmbH, Baxalta Incorporated. (Dudash, Amy) (Entered: 07/23/2018)
07/23/2018	<u>251</u>	Letter to The Honorable Timothy B. Dyk from Amy Dudash regarding the Confidential Preliminary Injunction Hearing Exhibits. (Attachments: # <u>1</u> Exhibit B)(Dudash, Amy) (Entered: 07/23/2018)
07/25/2018	<u>252</u>	REDACTED VERSION of <u>245</u> Letter, <i>regarding Preliminary Injunction Exhibits</i> by Baxalta GmbH, Baxalta Incorporated. (Attachments: # <u>1</u> Exhibit A – Joint Exhibit List, # <u>2</u> Exhibit Vol. 1 REDACTED, # <u>3</u> Exhibit Vol. 2 REDACTED, # <u>4</u> Exhibit Vol. 3 REDACTED, # <u>5</u> Exhibit Vol. 4 REDACTED, # <u>6</u> Exhibit Vol. 5 REDACTED)(Dudash, Amy) (Entered: 07/25/2018)
07/25/2018	<u>253</u>	REDACTED VERSION of <u>242</u> Statement [ <i>Response to Genentech's Post–Hearing Proposed Findings of Fact and Conclusions of Law</i> ] by Baxalta GmbH, Baxalta Incorporated. (Dudash, Amy) (Entered: 07/25/2018)
07/27/2018	<u>254</u>	NOTICE requesting Clerk to remove James V. Razick as co–counsel.. (Balick, Steven) (Entered: 07/27/2018)
07/30/2018	<u>255</u>	ORDER re <u>251</u> Letter to The Honorable Timothy B. Dyk from Amy Dudash regarding the Confidential Preliminary Injunction Hearing Exhibits. (SEE ORDER FOR FURTHER DETAILS). Signed by Judge Timothy Belcher Dyk on 7/26/2018. (crb) (Entered: 07/30/2018)
07/30/2018	<u>256</u>	ORDER and Commission for Depositions to be Taken in Japan. Signed by Judge Timothy Belcher Dyk on 7/25/2018. (crb) (Entered: 07/30/2018)
08/01/2018	<u>257</u>	Amended PROPOSED ORDER and Commission for Depositions to be Taken in Japan by Baxalta GmbH, Baxalta Incorporated. (Dudash, Amy) (Entered: 08/01/2018)
08/01/2018	<u>258</u>	[SEALED] Letter to The Honorable Timothy B. Dyk from Steven J. Balick regarding Response to the Court's July 30, 2018 Order – re <u>255</u> Order. (Balick, Steven) (Entered: 08/01/2018)
08/01/2018	<u>259</u>	[SEALED] DECLARATION re <u>258</u> Letter ( <i>Declaration of Gina Chapman in Support of Genentech's Request to Maintain Confidentiality of Certain Materials Containing Competitively Sensitive Information</i> ) by Genentech, Inc.. (Attachments: # <u>1</u> Exhibit A–B)(Balick, Steven) (Entered: 08/01/2018)
08/03/2018	<u>261</u>	Corrected ORDER and Commission for Depositions to be Taken in Japan. Signed by Judge Timothy Belcher Dyk on 8/3/2018. (nmg) (Entered: 08/06/2018)
08/06/2018	<u>260</u>	ANSWER to <u>240</u> Answer to Amended Complaint, Counterclaim [ <i>Plaintiffs' Answer to the Counterclaims of Defendant Genentech, Inc.</i> ] by Baxalta GmbH, Baxalta Incorporated.(Dudash, Amy) (Entered: 08/06/2018)
08/07/2018	<u>262</u>	OPINION & ORDER denying <u>41</u> MOTION for Preliminary Injunction Signed by Judge Timothy Belcher Dyk on 8/7/2018. (nmf) (Entered: 08/07/2018)

08/07/2018	<u>263</u>	NOTICE of Withdrawal of Appearance of Colm F. Connolly by Baxalta GmbH, Baxalta Incorporated (Barillare, Jody) (Entered: 08/07/2018)
08/08/2018	<u>264</u>	REDACTED VERSION of <u>258</u> Letter by Genentech, Inc.. (Balick, Steven) (Entered: 08/08/2018)
08/08/2018	<u>265</u>	REDACTED VERSION of <u>259</u> Declaration, ( <i>Declaration of Gina Chapman</i> ) by Genentech, Inc.. (Attachments: # <u>1</u> Exhibit A–B)(Balick, Steven) (Entered: 08/08/2018)
08/08/2018	<u>266</u>	NOTICE to Take Deposition of Friedrich Scheifflinger filed by Genentech, Inc..(Balick, Steven) (Entered: 08/08/2018)
08/08/2018	<u>267</u>	NOTICE to Take Deposition of Randolph Kerschbaumer filed by Genentech, Inc..(Balick, Steven) (Entered: 08/08/2018)
08/08/2018	<u>268</u>	NOTICE to Take Deposition of Thomas Larmondra filed by Genentech, Inc..(Balick, Steven) (Entered: 08/08/2018)
08/08/2018	<u>269</u>	NOTICE to Take Deposition of Noah Brown filed by Genentech, Inc..(Balick, Steven) (Entered: 08/08/2018)
08/08/2018	<u>270</u>	NOTICE to Take Deposition of Michael Moye filed by Genentech, Inc..(Balick, Steven) (Entered: 08/08/2018)
08/08/2018	<u>271</u>	NOTICE to Take Deposition of Gabi Gerstenbauer filed by Genentech, Inc..(Balick, Steven) (Entered: 08/08/2018)
08/08/2018	<u>272</u>	NOTICE to Take Deposition of Michael Dockal filed by Genentech, Inc..(Balick, Steven) (Entered: 08/08/2018)
08/08/2018	<u>273</u>	NOTICE OF SERVICE of (1) Defendant Genentech's Notice of Deposition of Baxalta Pursuant to Fed. R. Civ. P. 30(b)(6); (2) Genentech's Responses and Objections to Baxalta's 30(b)(6) Notice; and (3) Genentech, Inc.'s Fourth Set of Requests for the Production of Documents and Things to Baxalta Incorporated and Baxalta GmbH filed by Genentech, Inc..(Balick, Steven) (Entered: 08/08/2018)
08/09/2018	<u>274</u>	MOTION for Pro Hac Vice Appearance of Attorney Josephine Young, Naz Wehrli, Marissa C.M. Doran, and Alexander F. Atkins – filed by Genentech, Inc.. (Balick, Steven) (Entered: 08/09/2018)
08/10/2018	<u>275</u>	ORDER, The documents listed in Exhibit A to the parties' joint letter, ECF No. 254–1, are ADMITTED and are part of the preliminary injunction record. The parties' objections to inclusion of these documents are OVERRULED. Signed by Judge Timothy Belcher Dyk on 8/10/2018. (crb) (Entered: 08/10/2018)
08/15/2018	<u>276</u>	[SEALED] Letter to The Honorable Timothy B. Dyk from Steven J. Balick regarding Genentech's Request for an Order Compelling Baxalta to Respond to Discovery. (Attachments: # <u>1</u> Exhibit 1–10)(Balick, Steven) (Entered: 08/15/2018)
08/17/2018	<u>277</u>	NOTICE OF SERVICE of Plaintiffs' Responses and Objections to Genentech's Third Requests for Production of Documents and Things re <u>238</u> Notice of Service filed by Baxalta GmbH, Baxalta Incorporated.(Dudash, Amy) (Entered: 08/17/2018)
08/20/2018	<u>278</u>	ORDER granting <u>274</u> MOTION for Pro Hac Vice Appearance of Attorney Josephine Young, Naz Wehrli, Marissa C.M. Doran, and Alexander F. Atkins. Signed by Judge Timothy Belcher Dyk on 8/17/2018. (crb) (Entered: 08/20/2018)
08/21/2018		Pro Hac Vice Attorney Marissa C.M. Doran for Genentech, Inc. added for electronic noticing. Pursuant to Local Rule 83.5 (d)., Delaware counsel shall be the registered users of CM/ECF and shall be required to file all papers. (crb) (Entered: 08/21/2018)
08/21/2018		Pro Hac Vice Attorney Alexander F. Atkins for Genentech, Inc. added for electronic noticing. Pursuant to Local Rule 83.5 (d)., Delaware counsel shall be the registered users of CM/ECF and shall be required to file all papers. (crb) (Entered: 08/21/2018)
08/21/2018		Pro Hac Vice Attorney Josephine Young for Genentech, Inc. added for electronic noticing. Pursuant to Local Rule 83.5 (d)., Delaware counsel shall be the registered users of CM/ECF and shall be required to file all papers. (crb) (Entered: 08/21/2018)

08/21/2018		Pro Hac Vice Attorney Naz Wehrli for Genentech, Inc. added for electronic noticing. Pursuant to Local Rule 83.5 (d)., Delaware counsel shall be the registered users of CM/ECF and shall be required to file all papers. (crb) (Entered: 08/21/2018)
08/21/2018	<u>279</u>	NOTICE OF SERVICE of Genentech, Inc.'s Second Set of Interrogatories (Nos. 12–24) and Genentech, Inc.'s Second Set of Requests for Admission to Baxalta Incorporated and Baxalta GmbH filed by Genentech, Inc..(Mayo, Andrew) (Entered: 08/21/2018)
08/22/2018	<u>280</u>	[SEALED] Letter to Honorable Timothy B. Dyk from Amy Dudash regarding Opposition to Genentech's Request for an Order Compelling Baxalta to Respond to Discovery – re <u>276</u> Letter. (Attachments: # <u>1</u> Exhibit A)(Dudash, Amy) (Entered: 08/22/2018)
08/23/2018	<u>281</u>	NOTICE OF SERVICE of Notice of Deposition of Qasim Rizvi and Notice of Deposition of Cristin Hubbard filed by Baxalta GmbH, Baxalta Incorporated.(Dudash, Amy) (Entered: 08/23/2018)
08/23/2018	<u>282</u>	NOTICE OF SERVICE of Genentech, Inc.'s Fifth Set of Requests for the Production of Documents and Things to Baxalta Incorporated and Baxalta GmbH filed by Genentech, Inc.(Mayo, Andrew) Modified on 9/7/2018 (nmf). (Main Document 282 replaced on 9/7/2018) (nmf, ). (Entered: 08/23/2018)
08/23/2018	<u>283</u>	REDACTED VERSION of <u>276</u> Letter by Genentech, Inc.. (Attachments: # <u>1</u> Exhibit 1–10)(Mayo, Andrew) (Entered: 08/23/2018)
08/24/2018	<u>284</u>	NOTICE OF SERVICE of Plaintiffs' Second Set of Requests for Production of Documents and Things (Nos. 108–117) filed by Baxalta GmbH, Baxalta Incorporated.(Dudash, Amy) (Entered: 08/24/2018)
08/27/2018	<u>285</u>	NOTICE OF SERVICE of Defendant Genentech, Inc.'s First Amended Initial Disclosures Pursuant to Fed. R. Civ. P. 26(a)(1) filed by Genentech, Inc..(Mayo, Andrew) (Entered: 08/27/2018)
08/29/2018		ORAL ORDER: The parties should be prepared to argue the discovery dispute raised in parties letters dated August 15, 2018, ECF No. <u>276</u> , and August 22, 2018, ECF No. <u>280</u> , at the Markman hearing scheduled for Thursday, September 13, 2018, at 10:00AM in Courtroom 203 of the U.S. Court of Appeals for the Federal Circuit, Washington, DC. Ordered by Judge Timothy B. Dyk on 8/19/18. (crb) (Entered: 08/29/2018)
08/29/2018	<u>286</u>	REDACTED VERSION of <u>280</u> Letter by Baxalta GmbH, Baxalta Incorporated. (Attachments: # <u>1</u> Exhibit A)(Dudash, Amy) (Entered: 08/29/2018)
09/05/2018	<u>287</u>	NOTICE OF SERVICE of Plaintiffs' Second Set of Interrogatories (Nos. 21–27) filed by Baxalta GmbH, Baxalta Incorporated.(Dudash, Amy) (Entered: 09/05/2018)
09/05/2018	<u>288</u>	NOTICE OF SERVICE of Plaintiffs' Notices of Deposition Directed to Nancy Valente, Kate Skrable, Glenn Pierce, Bea Lavery, Gina Chapman, and Gallia Levy filed by Baxalta GmbH, Baxalta Incorporated.(Dudash, Amy) (Entered: 09/05/2018)
09/07/2018		CORRECTING ENTRY: Per request of counsel, the pdf of D.I. <u>282</u> has been replaced and the docket text corrected to reflect that this is the "Fifth" Set of Requests for the Production of Documents. (nmf) (Entered: 09/07/2018)
09/07/2018	<u>289</u>	NOTICE OF SERVICE of Plaintiffs' Responses and Objections to Genentech's Fourth Set of Requests for Production of Documents and Things (No. 108) filed by Baxalta GmbH, Baxalta Incorporated.(Dudash, Amy) (Entered: 09/07/2018)
09/07/2018	<u>290</u>	NOTICE OF SERVICE of Plaintiffs' Responses and Objections to Defendants' Notice of Deposition (Topics 1–45) filed by Baxalta GmbH, Baxalta Incorporated.(Dudash, Amy) (Entered: 09/07/2018)
09/12/2018		ORAL ORDER: The Markman Hearing set for September 13, 2018, is rescheduled for September 21, 2018, at 11:00AM in Courtroom 203 of the U.S. Court of Appeals for the Federal Circuit, Washington, DC. Ordered by Judge Timothy B. Dyk on 9/12/18. (nmg) (Entered: 09/12/2018)
09/13/2018	<u>291</u>	PROPOSED ORDER Dismissing Defendant Chugai Pharmaceutical Co., Ltd. [Stipulated] by Baxalta GmbH, Baxalta Incorporated. (Dudash, Amy) (Entered: 09/13/2018)

09/18/2018		Minute Entry for proceedings held before Judge Timothy Belcher Dyk – Status Conference held on 9/18/2018. (ceg) (Entered: 09/19/2018)
09/19/2018	<u>292</u>	Amended PROPOSED ORDER Dismissing Defendant Chugai Pharmaceutical Co., Ltd. <i>and Stipulation</i> by Baxalta GmbH, Baxalta Incorporated. (Dudash, Amy) (Entered: 09/19/2018)
09/19/2018		ORAL ORDER: The Markman hearing scheduled for 9/21/2018 is cancelled and will be rescheduled for a later date. Ordered by Judge Timothy Belcher Dyk on 9/19/2018. (ceg) (Entered: 09/19/2018)
09/19/2018	<u>293</u>	SO ORDERED, <u>292</u> First Amended Stipulation and Order Dismissing Defendant Chugai Pharmaceutical Co., Ltd. Signed by Judge Timothy Belcher Dyk on 9/19/2018. (ceg) (Entered: 09/19/2018)
09/20/2018	<u>294</u>	Official Transcript of the Status Teleconference held on 9/18/2018 before Judge Timothy B. Dyk. Court Reporter Carol Connolly. Transcript may be viewed at the court public terminal or purchased through the Court Reporter/Transcriber before the deadline for Release of Transcript Restriction. After that date it may be obtained through PACER. Redaction Request due 10/11/2018. Redacted Transcript Deadline set for 10/22/2018. Release of Transcript Restriction set for 12/19/2018. (nmg) (Entered: 09/20/2018)
09/20/2018	<u>295</u>	NOTICE OF SERVICE of Plaintiffs' Responses and Objections to Genentech, Inc.'s Second Set of Requests for Admission (Nos. 9–23) filed by Baxalta GmbH, Baxalta Incorporated.(Dudash, Amy) (Entered: 09/20/2018)
09/21/2018	<u>296</u>	NOTICE OF SERVICE of Plaintiffs' Responses and Objections to Genentech, Inc.'s Second Set of Interrogatories (Nos. 12–24) filed by Baxalta GmbH, Baxalta Incorporated.(Dudash, Amy) (Entered: 09/21/2018)
09/24/2018	<u>297</u>	NOTICE OF SERVICE of Genentech's Objections and Responses to Baxalta's Second Set of Requests for Production filed by Genentech, Inc..(Mayo, Andrew) (Entered: 09/24/2018)
09/24/2018	<u>298</u>	NOTICE to Take Deposition of Gabi Gerstenbauer on September 28, 2018 filed by Genentech, Inc..(Mayo, Andrew) (Entered: 09/24/2018)
09/24/2018	<u>299</u>	NOTICE OF SERVICE of Baxalta Incorporated and Baxalta GmbH Responses and Objections to Genetech, Inc.'s Fifth Set of Requests for the Production of Documents and Things filed by Baxalta GmbH, Baxalta Incorporated.(Dudash, Amy) (Entered: 09/24/2018)
09/25/2018		ORAL ORDER: The Markman hearing previously scheduled for September 21, 2018, is now rescheduled for October 16, 2018, at 10:00 a.m. in Courtroom 203 of the U.S. Court of Appeals for the Federal Circuit, Washington, DC. Ordered by Judge Timothy B. Dyk on 9/25/18. (ceg) (Entered: 09/25/2018)
09/26/2018	<u>300</u>	NOTICE to Take Deposition of Michael Moye on October 3, 2018 filed by Genentech, Inc..(Mayo, Andrew) (Entered: 09/26/2018)
09/26/2018	<u>301</u>	NOTICE to Take Deposition of Cristin Hubbard on October 2, 2018 filed by Baxalta GmbH, Baxalta Incorporated.(Dudash, Amy) (Entered: 09/26/2018)
10/01/2018	<u>302</u>	NOTICE OF SERVICE of Plaintiffs' Supplemental Responses and Objections to Genentech, Inc.'s Second Set of Interrogatories filed by Baxalta GmbH, Baxalta Incorporated.(Dudash, Amy) (Entered: 10/01/2018)
10/01/2018	<u>303</u>	NOTICE OF SERVICE of Genentech, Inc.'s Supplemental Objections and Responses to Baxalta's Interrogatory No. 13 filed by Genentech, Inc..(Mayo, Andrew) (Entered: 10/01/2018)
10/04/2018	<u>304</u>	Letter to The Honorable Timothy B. Dyk from Steven J. Balick regarding the Parties' Proposed Amendments to the Case Schedule (D.I. 174). (Balick, Steven) (Entered: 10/04/2018)
10/04/2018	<u>305</u>	STIPULATION Regarding the Parties' Proposed Amendments to the Case Schedule (D.I. 174), by Genentech, Inc.. (Balick, Steven) (Entered: 10/04/2018)
10/08/2018	<u>306</u>	NOTICE OF SERVICE of Genentech, Inc.'s Objections and Responses to Baxalta's Second Set of Interrogatories (Nos. 21–27) filed by Genentech, Inc..(Mayo, Andrew) (Entered: 10/08/2018)
10/12/2018	<u>307</u>	MOTION for Issuance of Letters Rogatory <i>Requiring the Testimony of Named Inventor Dr. Randolph Kerschbaumer (Unopposed)</i> – filed by Genentech, Inc.. (Attachments: # <u>1</u> Exhibit 1 & 2)(Mayo, Andrew) (Entered: 10/12/2018)

10/12/2018	<u>308</u>	Letter to The Honorable Timothy B. Dyk from Andrew C. Mayo regarding request that the Court: 1) deem Baxalta to have admitted certain Requests for Admission; and 2) compel Baxalta to respond to certain Interrogatories and Document Requests. (Attachments: # <u>1</u> Exhibit 1–12)(Mayo, Andrew) (Entered: 10/12/2018)
10/15/2018	<u>309</u>	NOTICE OF SERVICE of Plaintiffs' Supplemental Responses and Objections to Genentech, Inc.'s Interrogatory No. 11 filed by Baxalta GmbH, Baxalta Incorporated.(Dudash, Amy) (Entered: 10/15/2018)
10/16/2018		Minute Entry for proceedings held before Judge Timothy Belcher Dyk – Markman Hearing held on 10/16/2018. As stated on the record, Genentech's motion to compel discovery of Baxalta's 2015 collaboration agreement with Novimmune S.A. to research and develop bispecific antibodies for the treatment of hemophilia A using Novimmune's bispecific–antibody technology, and Baxalta's 2017 license agreement with Novimmune to develop and commercialize those bispecific antibodies is GRANTED, but is DENIED without prejudice as to experimental data and the royalty rates and compensation paid to Novimmune. (Court Reporter: Ronda Thomas of Epiq Court Reporting). (ceg) (Entered: 10/17/2018)
10/19/2018	<u>310</u>	[SEALED] Letter to Honorable Timothy B. Dyk from Amy Dudash regarding Response to Genentech's letter Motion to Compel – re <u>308</u> Letter,. (Attachments: # <u>1</u> Exhibit A [SEALED], # <u>2</u> Exhibit B [SEALED], # <u>3</u> Exhibit C [SEALED], # <u>4</u> Exhibit D [SEALED])(Dudash, Amy) (Entered: 10/19/2018)
10/19/2018	<u>311</u>	EXHIBIT re <u>310</u> Letter, [ <i>Exhibits E–H</i> ] by Baxalta GmbH, Baxalta Incorporated. (Attachments: # <u>1</u> Exhibit Exhibit F, # <u>2</u> Exhibit Exhibit G, # <u>3</u> Exhibit H)(Dudash, Amy) (Entered: 10/19/2018)
10/23/2018	<u>312</u>	Letter to Honorable Judge Timothy B. Dyk from Amy M. Dudash regarding regarding Case Schedule. (Dudash, Amy) (Entered: 10/23/2018)
10/23/2018	<u>313</u>	PROPOSED ORDER to Amend the Schedule [ <i>STIPULATED</i> ] by Baxalta Incorporated. (Dudash, Amy) (Entered: 10/23/2018)
10/23/2018	<u>314</u>	PROPOSED ORDER (Stipulated Protective Order), by Genentech, Inc.. (Balick, Steven) (Entered: 10/23/2018)
10/24/2018		ORAL ORDER: The parties are directed to attempt to resolve the discovery disputes reflected in their letters dated October 12, 2018 (ECF No. <u>308</u> ), and October 19, 2018 (ECF No. <u>310</u> ). The parties are to present any unresolved disputes at a telephonic status conference on November 2, 2018 at 11:00 a.m. Ordered by Judge Timothy B. Dyk on 10/24/2018. (*a status conference is set for 11/2/2018 at 11:00 a.m.) (ceg) (Entered: 10/24/2018)
10/26/2018	<u>315</u>	REDACTED VERSION of <u>310</u> Letter, <i>Response to Genentech's Letter Motion to Compel</i> by Baxalta GmbH. (Attachments: # <u>1</u> Exhibit A–D [REDACTED])(Dudash, Amy) (Entered: 10/26/2018)
10/29/2018	<u>316</u>	ORDER Approving D.I. <u>314</u> Stipulated Protective Order filed by Genentech, Inc. Signed by Judge Timothy Belcher Dyk on 10/26/2018. (nmg) (Entered: 10/29/2018)
10/30/2018	<u>317</u>	ORDER Setting Mediation Conferences: A Mediation Conference is set for 4/10/2019 at 10:00 AM in Courtroom 2B before Judge Mary Pat Thyng. SEE ORDER FOR DETAILS. Signed by Judge Mary Pat Thyng on 10/30/18. (cak) (Entered: 10/30/2018)
10/30/2018	<u>318</u>	NOTICE OF SERVICE of Genentech, Inc.'s Supplemental Objections and Responses to Baxalta's First Set of Interrogatories (Nos. 120) filed by Genentech, Inc..(Mayo, Andrew) (Entered: 10/30/2018)
11/01/2018	<u>319</u>	NOTICE OF SERVICE of Plaintiffs' Supplemental Responses and Objections to Genentech, Inc.'s Interrogatory Nos. 4 and 7 and Plaintiffs' Supplemental Responses and Objections to Genentech's Interrogatory Nos. 15, 16, and 21–23 filed by Baxalta GmbH, Baxalta Incorporated.(Dudash, Amy) (Entered: 11/01/2018)
11/02/2018	<u>320</u>	Official Transcript of Markman Hearing held on 10/16/2018 before Judge Timothy Dyk. Court Reporter/Transcriber Ronda J. Thomas, Telephone number 312–386–2000. Transcript may be viewed at the court public terminal or purchased through the Court Reporter/Transcriber before the deadline for Release of Transcript Restriction. After that date it may be obtained through PACER Redaction Request due 11/23/2018. Redacted Transcript Deadline set for 12/3/2018. Release of Transcript Restriction set for 1/31/2019. (crb) (Entered: 11/02/2018)

11/02/2018		ORAL ORDER: The telephonic status conference scheduled for November 2, 2018 at 11AM is canceled. Ordered by Judge Timothy B. Dyk on 11/2/2018. (crb) (Entered: 11/02/2018)
11/05/2018	<u>321</u>	NOTICE to Take Deposition of Gallia Levy on November 13, 2018 filed by Baxalta GmbH, Baxalta Incorporated.(Dudash, Amy) (Entered: 11/05/2018)
11/05/2018	<u>322</u>	NOTICE to Take Deposition of Gina Chapman on November 15, 2018 filed by Baxalta GmbH, Baxalta Incorporated.(Dudash, Amy) (Entered: 11/05/2018)
11/05/2018	<u>323</u>	NOTICE to Take Deposition of Kate Skrable on November 16, 2018 filed by Baxalta GmbH, Baxalta Incorporated.(Dudash, Amy) (Entered: 11/05/2018)
11/05/2018	<u>324</u>	NOTICE to Take Deposition of Qasim Rizvi on December 4, 2018 filed by Baxalta GmbH, Baxalta Incorporated.(Dudash, Amy) (Entered: 11/05/2018)
11/07/2018	<u>325</u>	ORDER Amending Schedule: Fact Discovery completed by 12/14/2018. Opening Expert Reports due by 1/18/2019. Rebuttal Expert Reports due by 2/15/2019. Reply Expert Reports due by 3/8/2019. Expert Discovery due by 4/19/2019. Motions for Summary Judgment due by 5/3/2019. Responses to Motions for Summary Judgment due by 5/17/2019. Reply to Motions for Summary Judgment due by 5/24/2019. Signed by Judge Timothy Belcher Dyk on 11/7/2018. (nmg) (Entered: 11/07/2018)
11/08/2018	<u>326</u>	NOTICE OF SERVICE of Letter Rogatory to Compel the Testimony of Dr. Randolph Kerschbaumer, filed by Genentech, Inc..(Balick, Steven) (Entered: 11/08/2018)
11/27/2018	<u>327</u>	NOTICE OF SERVICE of Plaintiffs' First Supplemental Responses and Objections to Genentech, Inc.'s Second Set of Requests for Admission (Nos. 10–13) filed by Baxalta Incorporated.(Dudash, Amy) (Entered: 11/27/2018)
11/27/2018	<u>328</u>	NOTICE OF SERVICE of Plaintiffs' Second Supplemental Response and Objections to Genentech, Inc.'s interrogatory No. 4 filed by Baxalta Incorporated.(Dudash, Amy) (Entered: 11/27/2018)
11/30/2018	<u>329</u>	NOTICE OF SERVICE of Plaintiffs' Second Supplemental Responses and Objections to Genentech, Inc.'s Interrogatory No. 11 filed by Baxalta GmbH, Baxalta Incorporated.(Dudash, Amy) (Entered: 11/30/2018)
12/03/2018	<u>330</u>	OPINION AND ORDER. Signed by Judge Timothy Belcher Dyk on 12/3/2018. (nmg) (Entered: 12/03/2018)
12/12/2018	<u>331</u>	STIPULATION Seeking to Amend Case Schedule, by Genentech, Inc.. (Balick, Steven) (Entered: 12/12/2018)
12/14/2018	<u>332</u>	STIPULATION Seeking to Amend Case Schedule, by Genentech, Inc.. (Balick, Steven) (Entered: 12/14/2018)
12/17/2018	<u>333</u>	Stipulation and Order to Amend the Schedule: ( Fact Discovery completed by 2/8/2019, Opening Expert Reports due by 3/15/2019, Rebuttal Expert Reports due by 4/12/2019, Expert Discovery due by 6/14/2019.) (See Order for Additional Deadlines). Signed by Judge Timothy Belcher Dyk on 12/17/2018. (nmg) (Entered: 12/17/2018)
01/25/2019	<u>334</u>	STIPULATION AND FINAL JUDGMENT, by Genentech, Inc.. (Balick, Steven) (Entered: 01/25/2019)
01/31/2019	<u>335</u>	STIPULATION AND FINAL JUDGMENT by Baxalta GmbH, Baxalta Incorporated. (Dudash, Amy) (Entered: 01/31/2019)
01/31/2019	<u>336</u>	Amended Disclosure Statement pursuant to Rule 7.1: identifying Other Affiliate Takeda Pharmaceutical Company Limited for Baxalta GmbH, Baxalta Incorporated filed by Baxalta GmbH, Baxalta Incorporated. (Dudash, Amy) (Entered: 01/31/2019)
02/01/2019	<u>337</u>	FINAL JUDGMENT is entered in favor of Genentech, Inc. and against Baxalta Incorporated on all claims asserted in the operative complaint. Genentech's counterclaims for declaratory judgment of non–infringement and a declaratory judgment of invalidity are dismissed without prejudice (*see Order for further details) (*CASE CLOSED). Signed by Judge Timothy Belcher Dyk on 1/31/2019. (ceg) (Entered: 02/01/2019)
02/01/2019	<u>338</u>	Report to the Commissioner of Patents and Trademarks for Patent/Trademark Number(s) 7,033,590. (Attachments: # <u>1</u> Approved Final Judgment)(ceg) (Entered: 02/01/2019)

02/08/2019	<u>339</u>	NOTICE OF APPEAL to the Federal Circuit of <u>330</u> Memorandum and Order, <u>337</u> Judgment, . Appeal filed by Baxalta GmbH, Baxalta Incorporated. (Dudash, Amy) (Entered: 02/08/2019)
02/08/2019		APPEAL – Credit Card Payment of \$505.00 received re <u>339</u> Notice of Appeal (Federal Circuit) filed by Baxalta GmbH, Baxalta Incorporated. ( Filing fee \$505, receipt number 0311–2565602.) (Dudash, Amy) (Entered: 02/08/2019)
02/08/2019		Notice of Appeal and Docket Sheet to US Court of Appeals for the Federal Circuit re <u>339</u> Notice of Appeal (Federal Circuit). (ddp) (Entered: 02/08/2019)
02/12/2019	<u>340</u>	NOTICE of Docketing Record on Appeal from USCA for the Federal Circuit re <u>339</u> Notice of Appeal (Federal Circuit) filed by Baxalta GmbH, Baxalta Incorporated. USCA Case Number 19–1527. Entry of Appearance due 02/26/2019. Certificate of Interest is due on 02/26/2019. Docketing Statement due 02/26/2019. Appellants' brief is due 04/15/2019. (bkb) (Entered: 02/12/2019)
02/13/2019	<u>341</u>	ORAL ORDER re <u>317</u> Order Setting Mediation Conferences: The mediation scheduled for 4/10/19 and the mediation submission due date of 3/25/19 are cancelled. Ordered by Judge Mary Pat Thyng on 2/13/19. (cak) (Entered: 02/13/2019)
02/20/2019	<u>342</u>	TRANSCRIPT REQUEST by Baxalta GmbH, Baxalta Incorporated for proceedings held on June 12, 2018, June 13, 2018, June 14, 2018, September 18, 2018, and October 16, 2018 before Judge Timothy Belcher Dyk, (Dudash, Amy) (Entered: 02/20/2019)
09/03/2019	<u>343</u>	MOTION (Genentech's Unopposed Motion to Perpetuate the Testimony of Austrian Citizen and Named Inventor Dr. Randolph Josef Kerschbaumer) – filed by Genentech, Inc.. (Attachments: # <u>1</u> Text of Proposed Order)(Balick, Steven) (Entered: 09/03/2019)
09/03/2019	<u>344</u>	[SEALED] MEMORANDUM in Support re <u>343</u> MOTION (Genentech's Unopposed Motion to Perpetuate the Testimony of Austrian Citizen and Named Inventor Dr. Randolph Josef Kerschbaumer) filed by Genentech, Inc.. Answering Brief/Response due date per Local Rules is 9/17/2019. (Attachments: # <u>1</u> Exhibit A–C)(Balick, Steven) (Entered: 09/03/2019)
09/03/2019	<u>345</u>	REDACTED VERSION of <u>344</u> MEMORANDUM in Support, by Genentech, Inc.. (Attachments: # <u>1</u> Exhibit A–C)(Balick, Steven) (Entered: 09/03/2019)
09/10/2019	<u>346</u>	ORDER granting <u>343</u> MOTION (Genentech's Unopposed Motion to Perpetuate the Testimony of Austrian Citizen and Named Inventor Dr. Randolph Josef Kerschbaumer). Signed by Judge Timothy Belcher Dyk on 9/10/2019. (lam) (Entered: 09/10/2019)
11/04/2019	<u>347</u>	MOTION (Genentech's Unopposed Motion for Issuance of a Letter Request to the Austrian District Court for Permission to Use a Stenographer During the Austrian Hearing to Take the Testimony of Named Inventor Dr. Randolph Josef Kerschbaumer) – filed by Genentech, Inc.. (Attachments: # <u>1</u> Exhibit A, # <u>2</u> Text of Proposed Order)(Balick, Steven) (Entered: 11/04/2019)
11/06/2019	<u>348</u>	ORDER granting <u>347</u> MOTION (Genentech's Unopposed Motion for Issuance of a Letter Request to the Austrian District Court for Permission to Use a Stenographer During the Austrian Hearing to Take the Testimony of Named Inventor Dr. Randolph Josef Kerschbaumer). The requested letter in revised form is available for pickup. Signed by Judge Timothy Belcher Dyk on 11/6/19. (lam) (Entered: 11/06/2019)
02/11/2020		ORDER The court has received an untranslated copy of the transcript from the deposition of Dr. Randolph Josef Kerschbaumer. The parties should advise as to how they would like to proceed. Signed by Judge Timothy Belcher Dyk on 2/11/2020. (lam) (Entered: 02/11/2020)
02/20/2020		ORDER Per the request of both parties, the court has transmitted the untranslated copy of the transcript from the deposition of Dr. Randolph Josef Kerschbaumer to counsel for Genentech. Signed by Judge Timothy Belcher Dyk on 2/20/2020. (lam) (Entered: 02/20/2020)
05/26/2020	<u>349</u>	NOTICE of Withdrawal of Appearance of Jesse T. Dyer as counsel for Plaintiffs by Baxalta GmbH, Baxalta Incorporated (Dudash, Amy) (Entered: 05/26/2020)
10/05/2020	<u>350</u>	MANDATE of USCA as to <u>339</u> Notice of Appeal (Federal Circuit) filed by Baxalta GmbH, Baxalta Incorporated. USCA Decision: Vacated and Remanded. (Attachments: # <u>1</u> Opinion, # <u>2</u> Judgment)(kmd) (Entered: 10/05/2020)
10/05/2020	<u>351</u>	NOTICE of of Withdrawal of Certain Pro Hac Vice Counsel by Baxalta GmbH, Baxalta Incorporated (Dudash, Amy) (Entered: 10/05/2020)



10/08/2020	<u>352</u>	ORAL ORDER: On August 27, 2020, the U.S. Court of Appeals for the Federal Circuit issued its decision vacating this court's judgment of noninfringement and remanding the case to this court. Baxalta Inc. v. Genentech, Inc., 972 F.3d 1341, 1349 (Fed. Cir. 2020). The Federal Circuit's mandate issued on October 5, 2020. ECF No. 350. Given the Federal Circuit's decision, a telephonic status conference is scheduled for October 14, 2020, at 2:00 PM. The parties should be prepared to discuss a new scheduling order and related matters. Counsel for plaintiffs shall initiate the call to chambers once all participants are on the line. Counsel shall also arrange for a court reporter. Ordered by Judge Timothy Belcher Dyk on 10/8/2020. (kmd) Modified on 10/8/2020 (kmd). (Entered: 10/08/2020)
10/13/2020	<u>353</u>	Joint Letter to Honorable Timothy B. Dyk from Amy M. Dudash regarding Scheduling for Case Going Forward – re 352 Oral Order,,,, Order Setting Teleconference,,,, (Dudash, Amy) (Entered: 10/13/2020)
10/14/2020	<u>354</u>	ORAL ORDER: As discussed during today's telephonic status conference, the parties shall file an updated proposed scheduling order on or before October 21, 2020. Ordered by Judge Timothy B. Dyk on October 14, 2020. (cna, ) (Entered: 10/14/2020)
10/21/2020	<u>355</u>	Joint PROPOSED ORDER Joint Proposed Scheduling Order re 354 Oral Order by Baxalta GmbH, Baxalta Incorporated. (Dudash, Amy) (Entered: 10/21/2020)
10/23/2020	<u>356</u>	SCHEDULING ORDER. Signed by Judge Timothy Belcher Dyk on 10/23/2020. (amf) (Entered: 10/23/2020)
11/11/2020	<u>357</u>	NOTICE OF SERVICE of Genentech, Inc.'s Third Set of Requests for Admission (Nos. 24–42) and Genentech, Inc.'s Third Set of Interrogatories (Nos. 25–27) filed by Genentech, Inc..(Mayo, Andrew) (Entered: 11/11/2020)
11/13/2020	<u>358</u>	NOTICE OF SERVICE of Genentech, Inc.'s Second Amended Initial Disclosures Pursuant to Fed. R. Civ. P. 26(a)(1) filed by Genentech, Inc..(Mayo, Andrew) (Entered: 11/13/2020)
11/20/2020	<u>359</u>	SUBPOENA filed by Baxalta GmbH, Baxalta Incorporated directed to Qasim Rizvi for December 16, 2020 at 9:00 AM Central (Dudash, Amy) (Entered: 11/20/2020)
11/24/2020	<u>360</u>	SUBPOENA Returned Executed as to Qasim Rizvi on 11/21/2020 (Dudash, Amy) (Entered: 11/24/2020)
11/25/2020	<u>361</u>	NOTICE OF SERVICE of Plaintiffs' Responses and Objections to Third Set of Requests for Admission (Nos. 24–42) and Responses and Objections to Third Set of Interrogatories (Nos. 25–27) filed by Baxalta GmbH, Baxalta Incorporated.(Dudash, Amy) (Entered: 11/25/2020)
11/25/2020	<u>362</u>	NOTICE OF SERVICE of Genentech, Inc.'s Amended Initial Disclosures Pursuant to Paragraph 3 of the Default Standard for Discovery and Genentech, Inc.'s Third Amended Initial Disclosures Pursuant to Fed. R. Civ. P. 26(a)(1) filed by Genentech, Inc..(Balick, Steven) (Entered: 11/25/2020)
11/30/2020	<u>363</u>	NOTICE OF SERVICE of Plaintiffs' First Amended Rule 26(a)(1) Initial Disclosures filed by Baxalta GmbH, Baxalta Incorporated.(Dudash, Amy) (Entered: 11/30/2020)
12/04/2020		Minute Entry for proceedings held before Judge Timothy Belcher Dyk – a Telephonic Status Conference held on 10/14/2020, at 2:00 PM (Call lasted for 12 minutes). (Court Reporter was present) (myr) (Entered: 12/04/2020)
12/07/2020	<u>364</u>	NOTICE OF SERVICE of Plaintiffs' (i) Supplemental Responses and Objections to Genentech Inc.'s Interrogatory Nos. 3, 7, 10 and 11 of Genentech's First Set of Interrogatories, (ii) Supplemental Responses and Objections to Genentech Inc.'s Interrogatory Nos. 15, 16, 19 and 21–24 of Genentech Inc.'s Second Set of Interrogatories, and (iii) First Supplemental Responses and Objections to Genentech Inc.'s Second Set of Requests for Admission (Nos. 14–23) filed by Baxalta GmbH, Baxalta Incorporated.(Dudash, Amy) (Entered: 12/07/2020)
12/08/2020	<u>365</u>	NOTICE OF SERVICE of Genentech, Inc.'s Sixth Set of Requests for the Production of Documents and Things (Request Nos. 113–117) filed by Genentech, Inc..(Balick, Steven) (Entered: 12/08/2020)
12/18/2020	<u>366</u>	Joint STIPULATION and Proposed Order Concerning the Protocol for Conducting Remote Depositions by Baxalta GmbH, Baxalta Incorporated. (Dudash, Amy) (Entered: 12/18/2020)
12/22/2020	<u>367</u>	STIPULATION AND ORDER Concerning the Protocol for Conducting Remote Depositions. Signed by Judge Timothy Belcher Dyk on 12/22/2020. (amf) (Entered: 12/22/2020)

01/06/2021	<u>368</u>	NOTICE to Take Deposition of David Chaudry on January 27, 2021 filed by Baxalta GmbH, Baxalta Incorporated.(Dudash, Amy) (Entered: 01/06/2021)
01/07/2021	<u>369</u>	NOTICE OF SERVICE of Plaintiffs' Responses and Objections to Genentech's Sixth Set of Requests for the Production of Documents and Things (Nos. 113–117) filed by Baxalta GmbH, Baxalta Incorporated.(Dudash, Amy) (Entered: 01/07/2021)
01/13/2021	<u>370</u>	NOTICE OF SERVICE of Plaintiffs' Second Supplemental Disclosure of Infringement Contentions filed by Baxalta GmbH, Baxalta Incorporated.(Dudash, Amy) (Entered: 01/13/2021)
01/14/2021	<u>371</u>	NOTICE to Take Deposition of Friedrich Dorner on January 28, 2021 filed by Genentech, Inc..(Balick, Steven) (Entered: 01/14/2021)
01/14/2021	<u>372</u>	NOTICE to Take Deposition of Rebecca Carty on February 10, 2021 filed by Genentech, Inc..(Balick, Steven) (Entered: 01/14/2021)
01/14/2021	<u>373</u>	NOTICE to Take Deposition of Friedrich Scheiflinger on February 17, 2021 filed by Genentech, Inc..(Balick, Steven) (Entered: 01/14/2021)
01/14/2021	<u>374</u>	NOTICE OF SERVICE of Genentech, Inc.'s Amended Notice of Rule 30(b)(6) Deposition to Baxalta Incorporated and Baxalta GmbH filed by Genentech, Inc..(Balick, Steven) (Entered: 01/14/2021)
01/18/2021	<u>375</u>	MOTION for Pro Hac Vice Appearance of Attorney Olga Berson, Ph.D. – filed by Baxalta GmbH, Baxalta Incorporated. (Attachments: # <u>1</u> Certification of Olga Berson, Ph.D.)(Dudash, Amy) (Entered: 01/18/2021)
01/19/2021	<u>376</u>	NOTICE to Take Deposition of Suha Patel on February 12, 2021 filed by Baxalta GmbH, Baxalta Incorporated.(Dudash, Amy) (Entered: 01/19/2021)
01/20/2021	<u>377</u>	NOTICE OF SERVICE of Plaintiffs' Third Set of Requests for the Production of Documents and Things (Nos. 117–138), Third Set of Interrogatories (No. 28), and Second Set of Requests for Admission Nos. 4–39) filed by Baxalta GmbH, Baxalta Incorporated.(Dudash, Amy) (Entered: 01/20/2021)
01/21/2021	<u>378</u>	NOTICE OF SERVICE of Genentech, Inc.'s Seventh Set of Requests for the Production of Documents and Things (Request No. 118) filed by Genentech, Inc..(Balick, Steven) (Entered: 01/21/2021)
01/21/2021	<u>379</u>	NOTICE OF SERVICE of Plaintiffs' Fourth Set of Interrogatories (Nos. 29–30) filed by Baxalta GmbH, Baxalta Incorporated.(Dudash, Amy) (Entered: 01/21/2021)
01/22/2021	<u>380</u>	SO ORDERED, re D.I. <u>375</u> MOTION for Pro Hac Vice Appearance of Attorney Olga Berson, Ph.D. filed by Baxalta GmbH, Baxalta Incorporated. Signed by Judge Timothy Belcher Dyk on 1/22/2021. (myr) (Entered: 01/22/2021)
01/25/2021		Pro Hac Vice Attorney Olga Berson for Baxalta GmbH and Baxalta Incorporated added for electronic noticing. Pursuant to Local Rule 83.5 (d)., Delaware counsel shall be the registered users of CM/ECF and shall be required to file all papers. (kmd) (Entered: 01/25/2021)
01/28/2021	<u>381</u>	NOTICE OF SERVICE of Plaintiffs' Second Supplemental Response to Second Set of Interrogatories (No. 24), Supplemental Response to First Set of Interrogatories (No. 10–11) and Response to Third Set of Interrogatories (No. 26) filed by Baxalta GmbH, Baxalta Incorporated.(Dudash, Amy) (Entered: 01/28/2021)
01/29/2021	<u>382</u>	NOTICE OF SERVICE of Genentech Inc.'s Supplemental Objections and Responses to Baxalta Incorporated's and Baxalta GmbH's Interrogatory Nos. 10, 23, and 24 filed by Genentech, Inc..(Mayo, Andrew) (Entered: 01/29/2021)
02/03/2021		ORAL ORDER, On February 2, 2021, the parties notified the court that they are at an impasse regarding various discovery disputes. It is hereby ORDERED that (1) defendants shall file an opening letter brief on or before Friday, February 5, 2021, addressing the disputes; and (2) plaintiffs shall file a responsive letter brief on or before Friday, February 12, 2021, addressing the disputes. The parties' letter briefs shall be no longer than seven pages, double spaced. It is further ORDERED that a telephonic hearing on the discovery disputes is scheduled for Wednesday, February 17, 2021, at 2:00 PM. Counsel for defendants shall initiate the call to chambers once all participants are on the line. Counsel for defendants shall also arrange for a court reporter. Entered by Judge Timothy Belcher Dyk on 2/3/2021. (nmg) (Entered: 02/03/2021)

		02/03/2021)
02/03/2021	<u>383</u>	NOTICE OF SERVICE of Defendant Genentech, Inc.'s Initial Non-Infringement Contentions and Defendant Genentech, Inc.'s First Supplemental Invalidity Contentions filed by Genentech, Inc..(Mayo, Andrew) (Entered: 02/03/2021)
02/05/2021	<u>384</u>	NOTICE OF SERVICE of Notice of Subpoenas filed by Genentech, Inc..(Mayo, Andrew) (Entered: 02/05/2021)
02/05/2021	<u>385</u>	[SEALED] Letter to The Honorable Timothy B. Dyk from Andrew C. Mayo regarding request that the Court compel Baxalta to supplement certain Interrogatories Responses and Renewed Request for Production of collaboration agreement with Novimmune S.A.. (Attachments: # <u>1</u> Exhibit A, # <u>2</u> Exhibit B, # <u>3</u> Exhibit C, # <u>4</u> Exhibit D, # <u>5</u> Exhibit E, # <u>6</u> Exhibit F, # <u>7</u> Exhibit G, # <u>8</u> Exhibit H, # <u>9</u> Exhibit I, # <u>10</u> Exhibit J)(Mayo, Andrew) (Entered: 02/05/2021)
02/12/2021	<u>386</u>	[SEALED] Letter to Honorable Timothy B. Dyk from Amy M. Dudash regarding pursuant to Court's February 3, 2021 Oral Order. (Attachments: # <u>1</u> Exhibit 1 SEALED, # <u>2</u> Exhibit 2 SEALED, # <u>3</u> Exhibit 3 SEALED, # <u>4</u> Exhibit 4 SEALED, # <u>5</u> Exhibit 5 SEALED, # <u>6</u> Exhibit 6 SEALED)(Dudash, Amy) (Entered: 02/12/2021)
02/12/2021	<u>387</u>	CERTIFICATE OF SERVICE of Plaintiffs' Letter and Exhibits 1–6 filed under seal [D.I. 386] by Baxalta GmbH, Baxalta Incorporated re <u>386</u> Letter, (Dudash, Amy) (Entered: 02/12/2021)
02/12/2021	<u>388</u>	REDACTED VERSION of <u>385</u> Letter, by Genentech, Inc.. (Attachments: # <u>1</u> Exhibit A–J)(Mayo, Andrew) (Entered: 02/12/2021)
02/17/2021	<u>389</u>	NOTICE OF SERVICE of Plaintiffs' Responses and Objections to Defendant's Amended Notice of 30(b)(6) Deposition (Topics 1–28) filed by Baxalta GmbH, Baxalta Incorporated.(Dudash, Amy) (Entered: 02/17/2021)
02/17/2021		Minute Entry for proceedings held before Judge Timothy Belcher Dyk – A Telephonic Hearing on the parties' discovery dispute held on February 17, 2021, at 2:00 PM. Baxalta Inc. ordered to supplement its response to Genentech, Inc.'s Interrogatory 9. Remaining relief requested denied without prejudice to the filing of a renewed motion at a later date. (Call lasted for 25 minutes) (Court Report was present) (myr) (Entered: 02/19/2021)
02/19/2021	<u>390</u>	REDACTED VERSION of <u>386</u> Letter, to Honorable Timothy B. Dyk by Baxalta GmbH, Baxalta Incorporated. (Attachments: # <u>1</u> Exhibit 1, # <u>2</u> Exhibit 2, # <u>3</u> Exhibit 3, # <u>4</u> Exhibit 4, # <u>5</u> Exhibit 5, # <u>6</u> Exhibit 6)(Dudash, Amy) (Entered: 02/19/2021)
02/22/2021	<u>391</u>	NOTICE OF SERVICE of Plaintiffs' Validity Contentions filed by Baxalta GmbH, Baxalta Incorporated.(Dudash, Amy) (Entered: 02/22/2021)
02/22/2021	<u>392</u>	NOTICE OF SERVICE of Plaintiffs' Second Supplemental Objections and Responses to Interrogatory No. 1, Fourth Supplemental Objections and Responses to Second Set of Interrogatories (Nos. 7, 9), Fourth Supplemental Objections and Responses to Third Set of Interrogatories (Nos. 21, 22, 23) and Objections and Response to Seventh Set of Requests for Production (No. 118) filed by Baxalta GmbH, Baxalta Incorporated.(Dudash, Amy) (Entered: 02/22/2021)
02/23/2021	<u>393</u>	NOTICE OF SERVICE of (1) Genentech's Objections and Responses to Baxalta's Second Set of Requests for Admission (Nos. 4–39); (2) Genentech, Inc.'s Objections and Responses to Baxalta's Fourth Set of Interrogatories (No. 29–30); (3) Genentech, Inc.'s Objections and Responses to Baxalta's Third Set of Interrogatories (No. 28); and (4) Genentech's Objections and Responses to Baxalta's Third Set of Requests for Production (Nos. 117–138) filed by Genentech, Inc..(Mayo, Andrew) (Entered: 02/23/2021)
02/23/2021	<u>394</u>	NOTICE OF SERVICE of Plaintiffs' Third Supplemental Disclosure of Infringement Contentions filed by Baxalta GmbH, Baxalta Incorporated.(Dudash, Amy) (Entered: 02/23/2021)
02/26/2021	<u>395</u>	NOTICE OF SERVICE of Genentech's Amended and Supplemental Responses and Objections to Baxalta's Rule 30(b)(6) Notice filed by Genentech, Inc..(Mayo, Andrew) (Entered: 02/26/2021)
03/11/2021	<u>396</u>	NOTICE OF SERVICE of (1) Defendant Genentech, Inc.'s Second Supplemental Invalidity Contentions; (2) Defendant Genentech, Inc.'s Supplemental Non-Infringement Contentions; and (3) Genentech Inc.'s Supplemental Objections and Responses to Baxalta Incorporated's and Baxalta GmbH's Second Set of Interrogatories (Nos. 21–23, 25 & 27) filed by Genentech,

		Inc..(Mayo, Andrew) (Entered: 03/11/2021)
03/11/2021	<u>397</u>	NOTICE OF SERVICE of Plaintiffs' Supplemental Responses and Objections to First Set of Interrogatories (Nos. 3, 5, 7, 9–11); Supplemental Responses and Objections to Second Set of Interrogatories (Nos. 12–20, 23–24); Supplemental Responses and Objections to Third Set of Interrogatories (Nos. 25, 27); Second Supplemental Responses and Objections to Second Set of Requests for Admission (Nos. 10, 11, 13); First Supplemental Responses and Objections to Third Set of Requests for Admission (Nos. 24, 31) and Fourth Supplemental Disclosure of Infringement Contentions filed by Baxalta GmbH, Baxalta Incorporated.(Dudash, Amy) (Entered: 03/11/2021)
03/11/2021	<u>398</u>	NOTICE OF SERVICE of Genentech, Inc.'s Supplemental Objections and Responses to Baxalta Incorporated's and Baxalta GmbH's First Set of Interrogatories (Nos. 3, 8, 11, 12 & 15–20) filed by Genentech, Inc..(Mayo, Andrew) (Entered: 03/11/2021)
04/02/2021	<u>399</u>	STIPULATION Regarding Supplementation of Protective Order, by Genentech, Inc.. (Balick, Steven) (Entered: 04/02/2021)
04/05/2021	<u>400</u>	ORDER Approving D.I. <u>399</u> Supplementation of Protective Order (Supplementing D.I. <u>316</u> ). Signed by Judge Timothy Belcher Dyk on 4/5/2021. (kmd) (Entered: 04/05/2021)
07/12/2021	<u>401</u>	MOTION for Pro Hac Vice Appearance of Attorney Wan–Shon Lo – filed by Baxalta GmbH, Baxalta Incorporated. (Attachments: # <u>1</u> Certification)(Dudash, Amy) (Entered: 07/12/2021)
07/12/2021	<u>402</u>	ORDER granting <u>401</u> MOTION for Pro Hac Vice Appearance of Attorney Wan–Shon Lo. Signed by Judge Timothy Belcher Dyk on 7/12/2021. (nmg) (Entered: 07/12/2021)
07/13/2021		Pro Hac Vice Attorney Wan–Shon Lo for Baxalta GmbH, and for Baxalta Incorporated added for electronic noticing. Pursuant to Local Rule 83.5 (d)., Delaware counsel shall be the registered users of CM/ECF and shall be required to file all papers. (twk) (Entered: 07/13/2021)
07/23/2021	<u>403</u>	MOTION for Pro Hac Vice Appearance of Attorney Catherine Nyarady – filed by Genentech, Inc.. (Balick, Steven) (Entered: 07/23/2021)
07/27/2021	<u>404</u>	ORDER, granting D.I. <u>403</u> Motion to Appear Pro Hac Vice Appearance of Attorney Catherine Nyarady. Signed by Judge Timothy Belcher Dyk on 7/27/2021. (myr) (Entered: 07/27/2021)
07/28/2021		Pro Hac Vice Attorney Catherine Nyarady for Genentech, Inc. added for electronic noticing. Pursuant to Local Rule 83.5 (d)., Delaware counsel shall be the registered users of CM/ECF and shall be required to file all papers. (twk) (Entered: 07/28/2021)
08/24/2021	<u>405</u>	STIPULATION Regarding Briefing of Dispositive Motions, by Genentech, Inc.. (Balick, Steven) (Entered: 08/24/2021)
08/25/2021	<u>406</u>	STIPULATION AND ORDER re D.I. <u>405</u> . Signed by Judge Timothy Belcher Dyk on 08/25/2021. (smg) (Entered: 08/25/2021)
09/03/2021	<u>407</u>	MOTION for Summary Judgment – filed by Genentech, Inc.. (Attachments: # <u>1</u> Text of Proposed Order)(Balick, Steven) (Entered: 09/03/2021)
09/03/2021	<u>408</u>	[SEALED] OPENING BRIEF in Support re <u>407</u> MOTION for Summary Judgment filed by Genentech, Inc..Answering Brief/Response due date per Local Rules is 9/17/2021. (Balick, Steven) (Entered: 09/03/2021)
09/03/2021	<u>409</u>	[SEALED] DECLARATION re <u>408</u> Opening Brief in Support ( <i>Declaration of David E. Cole, Volume 1 of 2</i> ) by Genentech, Inc.. (Attachments: # <u>1</u> Exhibit 1–14 (Volume 1 of 2))(Balick, Steven) (Entered: 09/03/2021)
09/03/2021	<u>410</u>	[SEALED] DECLARATION re <u>408</u> Opening Brief in Support ( <i>Declaration of David E. Cole, Volume 2 of 2</i> ) by Genentech, Inc.. (Attachments: # <u>1</u> Exhibit 15–28 (Volume 2 of 2))(Balick, Steven) (Entered: 09/03/2021)
09/03/2021	<u>411</u>	[SEALED] DECLARATION re <u>408</u> Opening Brief in Support ( <i>Declaration of John P. Sheehan, M.D.</i> ) by Genentech, Inc.. (Attachments: # <u>1</u> Exhibit 1–2)(Balick, Steven) (Entered: 09/03/2021)
09/03/2021	<u>412</u>	[SEALED] DECLARATION re <u>408</u> Opening Brief in Support ( <i>Declaration of Stephanie A. Smith, D.V.M., M.S.</i> ) by Genentech, Inc.. (Attachments: # <u>1</u> Exhibit 1–2)(Balick, Steven) (Entered: 09/03/2021)

09/03/2021	<u>413</u>	[SEALED] DECLARATION re <u>408</u> Opening Brief in Support ( <i>Declaration of William R. Strohl, Ph.D.</i> ) by Genentech, Inc.. (Attachments: # <u>1</u> Exhibit 1–3)(Balick, Steven) (Entered: 09/03/2021)
09/03/2021	<u>414</u>	[SEALED] DECLARATION re <u>408</u> Opening Brief in Support ( <i>Declaration of Guy A. Young, M.D.</i> ) by Genentech, Inc.. (Attachments: # <u>1</u> Exhibit 1)(Balick, Steven) (Entered: 09/03/2021)
09/03/2021	<u>415</u>	[SEALED] DECLARATION re <u>408</u> Opening Brief in Support ( <i>Declaration of Dr. K. Christopher Garcia</i> ) by Genentech, Inc.. (Attachments: # <u>1</u> Exhibit 1–2)(Balick, Steven) (Entered: 09/03/2021)
09/10/2021	<u>416</u>	REDACTED VERSION of <u>408</u> Opening Brief in Support by Genentech, Inc.. (Mayo, Andrew) (Entered: 09/10/2021)
09/10/2021	<u>417</u>	REDACTED VERSION of <u>409</u> Declaration of <i>David E. Cole (Volume 1 of 2)</i> by Genentech, Inc.. (Mayo, Andrew) (Entered: 09/10/2021)
09/10/2021	<u>418</u>	REDACTED VERSION of <u>410</u> Declaration of <i>David E. Cole (Volume 2 of 2)</i> by Genentech, Inc.. (Mayo, Andrew) (Entered: 09/10/2021)
09/10/2021	<u>419</u>	REDACTED VERSION of <u>411</u> Declaration of <i>John P. Sheehan, M.D.</i> by Genentech, Inc.. (Mayo, Andrew) (Entered: 09/10/2021)
09/10/2021	<u>420</u>	REDACTED VERSION of <u>412</u> Declaration of <i>Stephanie A. Smith, D.V.M., M.S.</i> by Genentech, Inc.. (Mayo, Andrew) (Entered: 09/10/2021)
09/10/2021	<u>421</u>	REDACTED VERSION of <u>413</u> Declaration of <i>William R. Strohl, Ph.D.</i> by Genentech, Inc.. (Mayo, Andrew) (Entered: 09/10/2021)
09/10/2021	<u>422</u>	REDACTED VERSION of <u>414</u> Declaration of <i>Guy A. Young, M.D.</i> by Genentech, Inc.. (Mayo, Andrew) (Entered: 09/10/2021)
09/10/2021	<u>423</u>	REDACTED VERSION of <u>415</u> Declaration of <i>Dr. K. Christopher Garcia</i> by Genentech, Inc.. (Mayo, Andrew) (Entered: 09/10/2021)
10/01/2021	<u>424</u>	[SEALED] ANSWERING BRIEF in Opposition re <u>407</u> MOTION for Summary Judgment filed by Baxalta GmbH, Baxalta Incorporated.Reply Brief due date per Local Rules is 10/8/2021. (Attachments: # <u>1</u> Index of Exhibits, # <u>2</u> Exhibit 1, # <u>3</u> Exhibit 2, # <u>4</u> Exhibit 3, # <u>5</u> Exhibit 4, # <u>6</u> Exhibit 5, # <u>7</u> Exhibit 6, # <u>8</u> Exhibit 7, # <u>9</u> Exhibit 8, # <u>10</u> Exhibit 9, # <u>11</u> Exhibit 10, # <u>12</u> Exhibit 11, # <u>13</u> Exhibit 12, # <u>14</u> Exhibit 13, # <u>15</u> Exhibit 14, # <u>16</u> Exhibit 15, # <u>17</u> Exhibit 16, # <u>18</u> Exhibit 17, # <u>19</u> Exhibit 18, # <u>20</u> Exhibit 19, # <u>21</u> Exhibit 20, # <u>22</u> Exhibit 21, # <u>23</u> Exhibit 22, # <u>24</u> Exhibit 23, # <u>25</u> Exhibit 24, # <u>26</u> Exhibit 25, # <u>27</u> Exhibit 26, # <u>28</u> Exhibit 27, # <u>29</u> Exhibit 28, # <u>30</u> Certificate of Service)(Dudash, Amy) (Entered: 10/01/2021)
10/15/2021	<u>425</u>	[SEALED] REPLY BRIEF re <u>407</u> MOTION for Summary Judgment filed by Genentech, Inc.. (Balick, Steven) (Entered: 10/15/2021)
10/22/2021	<u>426</u>	REDACTED VERSION of <u>425</u> Reply Brief by Genentech, Inc.. (Mayo, Andrew) (Entered: 10/22/2021)
10/27/2021	<u>427</u>	REDACTED VERSION of <u>424</u> Answering Brief in Opposition,,, by Baxalta GmbH, Baxalta Incorporated. (Attachments: # <u>1</u> Exhibit 1, # <u>2</u> Exhibit 2, # <u>3</u> Exhibit 3, # <u>4</u> Exhibit 4, # <u>5</u> Exhibit 5, # <u>6</u> Exhibit 6, # <u>7</u> Exhibit 7, # <u>8</u> Exhibit 8, # <u>9</u> Exhibit 9, # <u>10</u> Exhibit 10, # <u>11</u> Exhibit 11, # <u>12</u> Exhibit 12, # <u>13</u> Exhibit 13, # <u>14</u> Exhibit 14, # <u>15</u> Exhibit 15, # <u>16</u> Exhibit 16, # <u>17</u> Exhibit 17, # <u>18</u> Exhibit 18, # <u>19</u> Exhibit 19, # <u>20</u> Exhibit 20, # <u>21</u> Exhibit 21, # <u>22</u> Exhibit 22, # <u>23</u> Exhibit 23, # <u>24</u> Exhibit 24, # <u>25</u> Exhibit 25, # <u>26</u> Exhibit 26, # <u>27</u> Exhibit 27, # <u>28</u> Exhibit 28)(Dudash, Amy) (Entered: 10/27/2021)
10/27/2021	<u>428</u>	MOTION for Pro Hac Vice Appearance of Attorney William R. Peterson – filed by Baxalta GmbH, Baxalta Incorporated. (Attachments: # <u>1</u> Certification by Counsel to be Admitted Pro Hac Vice)(Dudash, Amy) (Entered: 10/27/2021)
10/29/2021		SO ORDERED, re <u>428</u> MOTION for Pro Hac Vice Appearance of Attorney William R. Peterson filed by Baxalta GmbH, Baxalta Incorporated. Signed by Judge Timothy Belcher Dyk on 10/29/2021. (myr) (Entered: 10/29/2021)
10/29/2021		Pro Hac Vice Attorney William R. Peterson for Baxalta GmbH and Baxalta Incorporated added for electronic noticing. Pursuant to Local Rule 83.5 (d)., Delaware counsel shall be the registered users of CM/ECF and shall be required to file all papers. (twk) (Entered: 10/29/2021)

11/18/2021	<u>429</u>	MOTION to Seal <i>Courtroom and Transcript Regarding Summary Judgment Hearing (UNOPPOSED)</i> – filed by Genentech, Inc.. (Balick, Steven) (Entered: 11/18/2021)
11/18/2021	<u>430</u>	[SEALED] MEMORANDUM in Support re <u>429</u> MOTION to Seal <i>Courtroom and Transcript Regarding Summary Judgment Hearing (UNOPPOSED)</i> filed by Genentech, Inc.. Answering Brief/Response due date per Local Rules is 12/2/2021. (Balick, Steven) (Entered: 11/18/2021)
11/22/2021		Minute Entry for proceedings held before Judge Timothy Belcher Dyk. Motion Hearing re <u>407</u> Motion for Summary Judgment held on 11/19/2021, at 10:00 AM. As stated on the record, <u>429</u> Genentechs Motion to Seal is DENIED. Parties to file stipulation by 12/03/21. (Court reporter was present) (nmg) (Entered: 11/22/2021)
11/24/2021	<u>431</u>	Official Transcript of Hearing held on 11/19/2021 before Judge Timothy B. Dyk. Transcript may be viewed at the court public terminal or order/purchased through the Court Reporter/Transcriber before the deadline for Release of Transcript Restriction. After that date, it may be obtained through PACER. Redaction Request due 12/15/2021. Redacted Transcript Deadline set for 12/27/2021. Release of Transcript Restriction set for 2/22/2022. (cna, ) (Entered: 11/29/2021)
11/29/2021	<u>432</u>	REDACTED VERSION of <u>430</u> MEMORANDUM in Support, by Genentech, Inc.. (Mayo, Andrew) (Entered: 11/29/2021)
12/01/2021	<u>433</u>	[SEALED] Proposed Pretrial Order by Baxalta GmbH, Baxalta Incorporated. (Attachments: # <u>1</u> Exhibit 1, # <u>2</u> Exhibit 2, # <u>3</u> Exhibit 3, # <u>4</u> Exhibit 4, # <u>5</u> Exhibit 5, # <u>6</u> Exhibit 6)(Dudash, Amy) (Entered: 12/01/2021)
12/01/2021	<u>434</u>	CERTIFICATE OF SERVICE of Proposed Final Pretrial Order and Exhibits 1–6 by Baxalta GmbH, Baxalta Incorporated re <u>433</u> Proposed Pretrial Order (Dudash, Amy) (Entered: 12/01/2021)
12/02/2021	<u>435</u>	MOTION to Seal ( <i>Unopposed Renewed and Narrowed Motion to Seal Portions of Summary Judgment Hearing Transcript</i> ) – filed by Genentech, Inc.. (Balick, Steven) (Entered: 12/02/2021)
12/02/2021	<u>436</u>	[SEALED] MEMORANDUM in Support re <u>435</u> MOTION to Seal ( <i>Unopposed Renewed and Narrowed Motion to Seal Portions of Summary Judgment Hearing Transcript</i> ) filed by Genentech, Inc.. Answering Brief/Response due date per Local Rules is 12/16/2021. (Attachments: # <u>1</u> Exhibit A)(Balick, Steven) (Entered: 12/02/2021)
12/03/2021	<u>437</u>	STIPULATION of Fact Regarding Hybridoma Technology and the Number of Anti–Factor IX/IXa Antibodies Disclosed In The '590 Patent, by Genentech, Inc.. (Balick, Steven) (Entered: 12/03/2021)
12/08/2021	<u>438</u>	[SEALED] MOTION in Limine <i>to Exclude Evidence of Post–Priority Development Efforts</i> – filed by Baxalta GmbH, Baxalta Incorporated. (Attachments: # <u>1</u> Exhibit 1, # <u>2</u> Exhibit 2, # <u>3</u> Exhibit 3, # <u>4</u> Exhibit 4, # <u>5</u> Exhibit 5, # <u>6</u> Exhibit 6, # <u>7</u> Exhibit 7, # <u>8</u> Exhibit 8, # <u>9</u> Text of Proposed Order, # <u>10</u> Certificate of Service)(Dudash, Amy) (Entered: 12/08/2021)
12/08/2021	<u>439</u>	[SEALED] MOTION in Limine <i>No. 2 to Exclude John P. Sheehan, M.D. and William R. Strohl Reliance on Confidential Documents for Written Description, Enablement and Indefiniteness</i> – filed by Baxalta GmbH, Baxalta Incorporated. (Attachments: # <u>1</u> Exhibit 1, # <u>2</u> Exhibit 2, # <u>3</u> Exhibit 3, # <u>4</u> Exhibit 4, # <u>5</u> Text of Proposed Order, # <u>6</u> Certificate of Service)(Dudash, Amy) (Entered: 12/08/2021)
12/08/2021	<u>440</u>	[SEALED] MOTION in Limine <i>No. 3 to Exclude Certain Evidence of Japanese Proceedings Concerning the Japanese Counterpart of the Patent–in–Suit</i> – filed by Baxalta GmbH, Baxalta Incorporated. (Attachments: # <u>1</u> Exhibit A, # <u>2</u> Exhibit B, # <u>3</u> Exhibit C, # <u>4</u> Exhibit D, # <u>5</u> Exhibit E, # <u>6</u> Exhibit F, # <u>7</u> Exhibit G, # <u>8</u> Exhibit H, # <u>9</u> Text of Proposed Order, # <u>10</u> Certificate of Service)(Dudash, Amy) (Entered: 12/08/2021)
12/08/2021	<u>441</u>	[SEALED] MOTION in Limine ( <i>Genentech, Inc.'s Motion In Limine No. 1 Regarding Literal Infringement</i> ) – filed by Genentech, Inc.. (Balick, Steven) (Entered: 12/08/2021)
12/08/2021	<u>442</u>	[SEALED] MOTION in Limine <i>No. 4 to Exclude Any Genentech, Chugai, and Roche Patents Relating to Hemlibra</i> – filed by Baxalta GmbH, Baxalta Incorporated. (Attachments: # <u>1</u> Exhibit 1, # <u>2</u> Exhibit 2, # <u>3</u> Exhibit 3, # <u>4</u> Text of Proposed Order, # <u>5</u> Certificate of Service)(Dudash, Amy) (Entered: 12/08/2021)

12/08/2021	<u>443</u>	[SEALED] MOTION in Limine ( <i>Genentech, Inc.'s Motion In Limine No. 2 to Preclude Improper Evidence of Willfulness</i> ) – filed by Genentech, Inc.. (Balick, Steven) (Entered: 12/08/2021)
12/08/2021	<u>444</u>	[SEALED] MOTION in Limine ( <i>Genentech, Inc.'s Motion In Limine No. 3 to Preclude Named Inventor Dr. Scheiflinger From Offering Expert Opinions</i> ) – filed by Genentech, Inc.. (Balick, Steven) (Entered: 12/08/2021)
12/08/2021	<u>445</u>	[SEALED] MOTION in Limine ( <i>Genentech, Inc.'s Motion In Limine No. 4 to Preclude Baxalta From Offering Written–Description Testimony that Factor X is an Obvious Choice for a Second Antigen for a Bispecific Antibody</i> ) – filed by Genentech, Inc.. (Balick, Steven) (Entered: 12/08/2021)
12/08/2021	<u>446</u>	[SEALED] MOTION in Limine ( <i>Genentech, Inc.'s Motion In Limine No. 5 to Preclude Baxalta From Presenting Arguments and Evidence Regarding Certain Deposited Hybridomas</i> ) – filed by Genentech, Inc.. (Balick, Steven) (Entered: 12/08/2021)
12/08/2021	<u>447</u>	[SEALED] MOTION in Limine ( <i>Genentech, Inc.'s Motion In Limine No. 6 to Preclude Baxalta From Offering Testimony Regarding scFv Derivatives</i> ) – filed by Genentech, Inc.. (Balick, Steven) (Entered: 12/08/2021)
12/08/2021	<u>448</u>	[SEALED] MOTION to Preclude ( <i>Genentech, Inc.'s Daubert Motion to Exclude Certain Opinions of Baxaltas Expert Leonard Paul Freedman</i> ) – filed by Genentech, Inc.. (Balick, Steven) (Entered: 12/08/2021)
12/08/2021	<u>449</u>	[SEALED] MOTION in Limine No. 5 to Preclude Genentech from Introducing Evidence or Testimony Relating to Antibodies Falling Outside the Scope of the Claims to Support Its Written Description Defense – filed by Baxalta GmbH, Baxalta Incorporated. (Attachments: # <u>1</u> Exhibit 1, # <u>2</u> Exhibit 2, # <u>3</u> Text of Proposed Order, # <u>4</u> Certificate of Service)(Dudash, Amy) (Entered: 12/08/2021)
12/08/2021	<u>450</u>	MOTION to Preclude ( <i>Genentech, Inc.'s Daubert Motion to Exclude the Opinions of Baxalta's Expert Witness Dr. Jeffrey J. Gray and Related Opinions of Baxalta's Expert Witness Dr. Wayne A. Marasco</i> ) – filed by Genentech, Inc.. (Balick, Steven) (Entered: 12/08/2021)
12/08/2021	<u>451</u>	[SEALED] MOTION to Preclude ( <i>Genentech, Inc.'s Daubert Motion to Exclude Certain Opinions of Baxalta's Expert Witness Dr. Sriram Krishnaswamy</i> ) – filed by Genentech, Inc.. (Balick, Steven) (Entered: 12/08/2021)
12/08/2021	<u>452</u>	[SEALED] MOTION to Preclude ( <i>Genentech, Inc.'s Daubert Motion to Exclude Certain Opinions of Baxalta's Expert James E. Malackowski</i> ) – filed by Genentech, Inc.. (Balick, Steven) (Entered: 12/08/2021)
12/08/2021	<u>453</u>	[SEALED] MOTION to Preclude ( <i>Genentech, Inc.'s Daubert Motion to Exclude Certain Opinions of Baxalta's Expert Dr. Wayne A. Marasco</i> ) – filed by Genentech, Inc.. (Balick, Steven) (Entered: 12/08/2021)
12/08/2021	<u>454</u>	[SEALED] DECLARATION ( <i>Declaration of David E. Cole in Support of Genentech, Inc.'s Motions In Limine and Daubert Motions, Volume I of II</i> ) by Genentech, Inc.. (Attachments: # <u>1</u> Exhibit 1–37)(Balick, Steven) (Entered: 12/08/2021)
12/08/2021	<u>455</u>	MOTION in Limine No. 6 to Exclude References to Plaintiffs' Motion for a Preliminary Injunction and Certain Related Materials – filed by Baxalta GmbH, Baxalta Incorporated. (Attachments: # <u>1</u> Text of Proposed Order)(Dudash, Amy) (Entered: 12/08/2021)
12/08/2021	<u>456</u>	[SEALED] DECLARATION ( <i>Declaration of David E. Cole in Support of Genentech, Inc.'s Motions In Limine and Daubert Motions, Volume II of II</i> ), by Genentech, Inc.. (Attachments: # <u>1</u> Exhibit 38–57)(Balick, Steven) (Entered: 12/08/2021)
12/08/2021	<u>457</u>	[SEALED] MOTION in Limine No. 7 to Preclude Genentech from Relying on the Court's Claim Construction Order to Refute Willfulness – filed by Baxalta GmbH, Baxalta Incorporated. (Attachments: # <u>1</u> Exhibit 1, # <u>2</u> Text of Proposed Order, # <u>3</u> Certificate of Service)(Dudash, Amy) (Entered: 12/08/2021)
12/08/2021	<u>458</u>	[SEALED] MOTION to Exclude Certain Opinions and Testimony of Drs. Steven Schwartz and Guy Young – filed by Baxalta GmbH, Baxalta Incorporated. (Attachments: # <u>1</u> Exhibit 1, # <u>2</u> Exhibit 2, # <u>3</u> Exhibit 3, # <u>4</u> Exhibit 4, # <u>5</u> Exhibit 5, # <u>6</u> Exhibit 6, # <u>7</u> Exhibit 7, # <u>8</u> Text of Proposed Order, # <u>9</u> Certificate of Service)(Dudash, Amy) (Entered: 12/08/2021)

12/08/2021	<u>459</u>	[SEALED] MOTION to Exclude Certain Opinions and Testimony of Dr. Richard Manning – filed by Baxalta GmbH, Baxalta Incorporated. (Attachments: # <u>1</u> Exhibit 1, # <u>2</u> Exhibit 2, # <u>3</u> Text of Proposed Order, # <u>4</u> Certificate of Service)(Dudash, Amy) (Entered: 12/08/2021)
12/08/2021	<u>460</u>	[SEALED] MOTION to Exclude Opinions and Testimony of Dr. Dougald M. Monroe, III – filed by Baxalta GmbH, Baxalta Incorporated. (Attachments: # <u>1</u> Exhibit A, # <u>2</u> Exhibit B, # <u>3</u> Exhibit C, # <u>4</u> Exhibit D, # <u>5</u> Exhibit E, # <u>6</u> Text of Proposed Order, # <u>7</u> Certificate of Service)(Dudash, Amy) (Entered: 12/08/2021)
12/08/2021	<u>461</u>	REDACTED VERSION of <u>433</u> Proposed Pretrial Order by Baxalta GmbH, Baxalta Incorporated. (Attachments: # <u>1</u> Exhibit 1, # <u>2</u> Exhibit 2, # <u>3</u> Exhibit 3, # <u>4</u> Exhibit 4, # <u>5</u> Exhibit 5, # <u>6</u> Exhibit 6)(Dudash, Amy) (Entered: 12/08/2021)
12/09/2021	<u>462</u>	REDACTED VERSION of <u>436</u> MEMORANDUM in Support, by Genentech, Inc.. (Balick, Steven) (Entered: 12/09/2021)
12/13/2021	<u>463</u>	Joint STIPULATION of Fact Regarding Hybridoma Technology and the Number of Anti-Factor IX/IXa Antibodies Disclosed In The '590 Patent. Signed by Judge Timothy Belcher Dyk on 12/13/2021. (nmg) (Entered: 12/13/2021)
12/15/2021	<u>464</u>	REDACTED VERSION of <u>441</u> MOTION in Limine ( <i>Genentech, Inc.'s Motion In Limine No. 1 Regarding Literal Infringement</i> ) by Genentech, Inc.. (Mayo, Andrew) (Entered: 12/15/2021)
12/15/2021	<u>465</u>	REDACTED VERSION of <u>443</u> MOTION in Limine ( <i>Genentech, Inc.'s Motion In Limine No. 2 to Preclude Improper Evidence of Willfulness</i> ) by Genentech, Inc.. (Mayo, Andrew) (Entered: 12/15/2021)
12/15/2021	<u>466</u>	REDACTED VERSION of <u>444</u> MOTION in Limine ( <i>Genentech, Inc.'s Motion In Limine No. 3 to Preclude Named Inventor Dr. Scheiflinger From Offering Expert Opinions</i> ) by Genentech, Inc.. (Mayo, Andrew) (Entered: 12/15/2021)
12/15/2021	<u>467</u>	REDACTED VERSION of <u>445</u> MOTION in Limine ( <i>Genentech, Inc.'s Motion In Limine No. 4 to Preclude Baxalta From Offering Written-Description Testimony that Factor X is an Obvious Choice for a Second Antigen for a Bispecific Antibody</i> ) by Genentech, Inc.. (Mayo, Andrew) (Entered: 12/15/2021)
12/15/2021	<u>468</u>	REDACTED VERSION of <u>446</u> MOTION in Limine ( <i>Genentech, Inc.'s Motion In Limine No. 5 to Preclude Baxalta From Presenting Arguments and Evidence Regarding Certain Deposited Hybridomas</i> ) by Genentech, Inc.. (Mayo, Andrew) (Entered: 12/15/2021)
12/15/2021	<u>469</u>	REDACTED VERSION of <u>447</u> MOTION in Limine ( <i>Genentech, Inc.'s Motion In Limine No. 6 to Preclude Baxalta From Offering Testimony Regarding scFv Derivatives</i> ) by Genentech, Inc.. (Mayo, Andrew) (Entered: 12/15/2021)
12/15/2021	<u>470</u>	REDACTED VERSION of <u>448</u> MOTION to Preclude ( <i>Genentech, Inc.'s Daubert Motion to Exclude Certain Opinions of Baxaltas Expert Leonard Paul Freedman</i> ) by Genentech, Inc.. (Mayo, Andrew) (Entered: 12/15/2021)
12/15/2021	<u>471</u>	REDACTED VERSION of <u>451</u> MOTION to Preclude ( <i>Genentech, Inc.'s Daubert Motion to Exclude Certain Opinions of Baxalta's Expert Witness Dr. Sriram Krishnaswamy</i> ) by Genentech, Inc.. (Mayo, Andrew) (Entered: 12/15/2021)
12/15/2021	<u>472</u>	REDACTED VERSION of <u>452</u> MOTION to Preclude ( <i>Genentech, Inc.'s Daubert Motion to Exclude Certain Opinions of Baxalta's Expert James E. Malackowski</i> ) by Genentech, Inc.. (Mayo, Andrew) (Entered: 12/15/2021)
12/15/2021	<u>473</u>	REDACTED VERSION of <u>453</u> MOTION to Preclude ( <i>Genentech, Inc.'s Daubert Motion to Exclude Certain Opinions of Baxalta's Expert Dr. Wayne A. Marasco</i> ) by Genentech, Inc.. (Mayo, Andrew) (Entered: 12/15/2021)
12/15/2021	<u>474</u>	REDACTED VERSION of <u>454</u> Declaration of David E. Cole in Support of Genentech, Inc.'s Motions In Limine and Daubert Motions (Volume I of II) by Genentech, Inc.. (Attachments: # <u>1</u> Exhibit 1–37)(Mayo, Andrew) (Entered: 12/15/2021)
12/15/2021	<u>475</u>	REDACTED VERSION of <u>456</u> Declaration of David E. Cole in Support of Genentech, Inc.'s Motions In Limine and Daubert Motions (Volume II of II) by Genentech, Inc.. (Attachments: # <u>1</u> Exhibit 38–57)(Mayo, Andrew) (Entered: 12/15/2021)



12/15/2021	<u>476</u>	REDACTED VERSION of <u>438</u> MOTION in Limine <i>to Exclude Evidence of Post–Priority Development Efforts</i> by Baxalta GmbH, Baxalta Incorporated. (Attachments: # <u>1</u> Exhibit 1, # <u>2</u> Exhibit 2, # <u>3</u> Exhibit 3, # <u>4</u> Exhibit 4, # <u>5</u> Exhibit 5, # <u>6</u> Exhibit 6, # <u>7</u> Exhibit 7, # <u>8</u> Exhibit 8, # <u>9</u> Text of Proposed Order)(Dudash, Amy) (Entered: 12/15/2021)
12/15/2021	<u>477</u>	REDACTED VERSION of <u>439</u> MOTION in Limine <i>No. 2 to Exclude John P. Sheehan, M.D. and William R. Strohl Reliance on Confidential Documents for Written Description, Enablement and Indefiniteness</i> by Baxalta GmbH, Baxalta Incorporated. (Attachments: # <u>1</u> Exhibit 1, # <u>2</u> Exhibit 2, # <u>3</u> Exhibit 3, # <u>4</u> Exhibit 4, # <u>5</u> Text of Proposed Order)(Dudash, Amy) (Entered: 12/15/2021)
12/15/2021	<u>478</u>	REDACTED VERSION of <u>440</u> MOTION in Limine <i>No. 3 to Exclude Certain Evidence of Japanese Proceedings Concerning the Japanese Counterpart of the Patent–in–Suit</i> by Baxalta GmbH, Baxalta Incorporated. (Attachments: # <u>1</u> Exhibit A, # <u>2</u> Exhibit B, # <u>3</u> Exhibit C, # <u>4</u> Exhibit D, # <u>5</u> Exhibit E, # <u>6</u> Exhibit F, # <u>7</u> Exhibit G, # <u>8</u> Exhibit H, # <u>9</u> Text of Proposed Order)(Dudash, Amy) (Entered: 12/15/2021)
12/15/2021	<u>479</u>	REDACTED VERSION of <u>442</u> MOTION in Limine <i>No. 4 to Exclude Any Genentech, Chugai, and Roche Patents Relating to Hemlibra</i> by Baxalta GmbH, Baxalta Incorporated. (Attachments: # <u>1</u> Exhibit 1, # <u>2</u> Exhibit 2, # <u>3</u> Exhibit 3, # <u>4</u> Text of Proposed Order)(Dudash, Amy) (Entered: 12/15/2021)
12/15/2021	<u>480</u>	REDACTED VERSION of <u>449</u> MOTION in Limine <i>No. 5 to Preclude Genentech from Introducing Evidence or Testimony Relating to Antibodies Falling Outside the Scope of the Claims to Support Its Written Description Defense</i> by Baxalta GmbH, Baxalta Incorporated. (Attachments: # <u>1</u> Exhibit 1, # <u>2</u> Exhibit 2, # <u>3</u> Text of Proposed Order)(Dudash, Amy) (Entered: 12/15/2021)
12/15/2021	<u>481</u>	REDACTED VERSION of <u>457</u> MOTION in Limine <i>No. 7 to Preclude Genentech from Relying on the Court's Claim Construction Order to Refute Willfulness</i> by Baxalta GmbH, Baxalta Incorporated. (Attachments: # <u>1</u> Exhibit 1, # <u>2</u> Text of Proposed Order)(Dudash, Amy) (Entered: 12/15/2021)
12/15/2021	<u>482</u>	REDACTED VERSION of <u>458</u> MOTION to Exclude Certain Opinions and Testimony of Drs. Steven Schwartz and Guy Young by Baxalta GmbH, Baxalta Incorporated. (Attachments: # <u>1</u> Exhibit 1, # <u>2</u> Exhibit 2, # <u>3</u> Exhibit 3, # <u>4</u> Exhibit 4, # <u>5</u> Exhibit 5, # <u>6</u> Exhibit 6, # <u>7</u> Exhibit 7, # <u>8</u> Text of Proposed Order)(Dudash, Amy) (Entered: 12/15/2021)
12/15/2021	<u>483</u>	REDACTED VERSION of <u>459</u> MOTION to Exclude Certain Opinions and Testimony of Dr. Richard Manning by Baxalta GmbH, Baxalta Incorporated. (Attachments: # <u>1</u> Exhibit 1, # <u>2</u> Exhibit 2, # <u>3</u> Text of Proposed Order)(Dudash, Amy) (Entered: 12/15/2021)
12/15/2021	<u>484</u>	REDACTED VERSION of <u>460</u> MOTION to Exclude Opinions and Testimony of Dr. Dougald M. Monroe, III by Baxalta GmbH, Baxalta Incorporated. (Attachments: # <u>1</u> Exhibit A, # <u>2</u> Exhibit B, # <u>3</u> Exhibit C, # <u>4</u> Exhibit D, # <u>5</u> Exhibit E, # <u>6</u> Text of Proposed Order)(Dudash, Amy) (Entered: 12/15/2021)
12/16/2021	<u>485</u>	[SEALED] ANSWERING BRIEF in Opposition re <u>441</u> MOTION in Limine ( <i>Genentech, Inc.'s Motion In Limine No. 1 Regarding Literal Infringement</i> ) filed by Baxalta GmbH, Baxalta Incorporated.Reply Brief due date per Local Rules is 12/27/2021. (Attachments: # <u>1</u> Exhibit 1, # <u>2</u> Exhibit 2, # <u>3</u> Exhibit 3, # <u>4</u> Exhibit 4, # <u>5</u> Certificate of Service)(Dudash, Amy) (Entered: 12/16/2021)
12/16/2021	<u>486</u>	[SEALED] ANSWERING BRIEF in Opposition re <u>443</u> MOTION in Limine ( <i>Genentech, Inc.'s Motion In Limine No. 2 to Preclude Improper Evidence of Willfulness</i> ) filed by Baxalta GmbH, Baxalta Incorporated.Reply Brief due date per Local Rules is 12/27/2021. (Attachments: # <u>1</u> Exhibit 1, # <u>2</u> Exhibit 2, # <u>3</u> Exhibit 3, # <u>4</u> Exhibit 4, # <u>5</u> Exhibit 5, # <u>6</u> Certificate of Service)(Dudash, Amy) (Entered: 12/16/2021)
12/16/2021	<u>487</u>	[SEALED] ANSWERING BRIEF in Opposition re <u>444</u> MOTION in Limine ( <i>Genentech, Inc.'s Motion In Limine No. 3 to Preclude Named Inventor Dr. Scheiflinger From Offering Expert Opinions</i> ) filed by Baxalta GmbH, Baxalta Incorporated.Reply Brief due date per Local Rules is 12/27/2021. (Attachments: # <u>1</u> Certificate of Service)(Dudash, Amy) (Entered: 12/16/2021)
12/16/2021	<u>488</u>	[SEALED] ANSWERING BRIEF in Opposition re <u>445</u> MOTION in Limine ( <i>Genentech, Inc.'s Motion In Limine No. 4 to Preclude Baxalta From Offering Written–Description Testimony that Factor X is an Obvious Choice for a Second Antigen for a Bispecific Antibody</i> ) filed by Baxalta GmbH, Baxalta Incorporated.Reply Brief due date per Local Rules is 12/27/2021. (Attachments:

		# <u>1</u> Certificate of Service)(Dudash, Amy) (Entered: 12/16/2021)
12/16/2021	<u>489</u>	[SEALED] ANSWERING BRIEF in Opposition re <u>438</u> MOTION in Limine <i>to Exclude Evidence of Post–Priority Development Efforts</i> filed by Genentech, Inc..Reply Brief due date per Local Rules is 12/27/2021. (Balick, Steven) (Entered: 12/16/2021)
12/16/2021	<u>490</u>	[SEALED] ANSWERING BRIEF in Opposition re <u>439</u> MOTION in Limine <i>No. 2 to Exclude John P. Sheehan, M.D. and William R. Strohl Reliance on Confidential Documents for Written Description, Enablement and Indefiniteness</i> filed by Genentech, Inc..Reply Brief due date per Local Rules is 12/27/2021. (Balick, Steven) (Entered: 12/16/2021)
12/16/2021	<u>491</u>	[SEALED] ANSWERING BRIEF in Opposition re <u>440</u> MOTION in Limine <i>No. 3 to Exclude Certain Evidence of Japanese Proceedings Concerning the Japanese Counterpart of the Patent–in–Suit</i> filed by Genentech, Inc..Reply Brief due date per Local Rules is 12/27/2021. (Balick, Steven) (Entered: 12/16/2021)
12/16/2021	<u>492</u>	[SEALED] ANSWERING BRIEF in Opposition re <u>446</u> MOTION in Limine ( <i>Genentech, Inc.'s Motion In Limine No. 5 to Preclude Baxalta From Presenting Arguments and Evidence Regarding Certain Deposited Hybridomas</i> ) filed by Baxalta GmbH, Baxalta Incorporated.Reply Brief due date per Local Rules is 12/27/2021. (Attachments: # <u>1</u> Exhibit 1, # <u>2</u> Exhibit 2, # <u>3</u> Exhibit 3, # <u>4</u> Exhibit 4, # <u>5</u> Exhibit 5, # <u>6</u> Exhibit 6, # <u>7</u> Exhibit 7, # <u>8</u> Exhibit 8, # <u>9</u> Exhibit 9, # <u>10</u> Exhibit 10, # <u>11</u> Certificate of Service)(Dudash, Amy) (Entered: 12/16/2021)
12/16/2021	<u>493</u>	[SEALED] ANSWERING BRIEF in Opposition re <u>442</u> MOTION in Limine <i>No. 4 to Exclude Any Genentech, Chugai, and Roche Patents Relating to Hemlibra</i> filed by Genentech, Inc..Reply Brief due date per Local Rules is 12/27/2021. (Balick, Steven) (Entered: 12/16/2021)
12/16/2021	<u>494</u>	[SEALED] ANSWERING BRIEF in Opposition re <u>449</u> MOTION in Limine <i>No. 5 to Preclude Genentech from Introducing Evidence or Testimony Relating to Antibodies Falling Outside the Scope of the Claims to Support Its Written Description Defense</i> filed by Genentech, Inc..Reply Brief due date per Local Rules is 12/27/2021. (Balick, Steven) (Entered: 12/16/2021)
12/16/2021	<u>495</u>	[SEALED] ANSWERING BRIEF in Opposition re <u>447</u> MOTION in Limine ( <i>Genentech, Inc.'s Motion In Limine No. 6 to Preclude Baxalta From Offering Testimony Regarding scFv Derivatives</i> ) filed by Baxalta GmbH, Baxalta Incorporated.Reply Brief due date per Local Rules is 12/27/2021. (Attachments: # <u>1</u> Exhibit 1, # <u>2</u> Exhibit 2, # <u>3</u> Exhibit 3, # <u>4</u> Certificate of Service)(Dudash, Amy) (Entered: 12/16/2021)
12/16/2021	<u>496</u>	ANSWERING BRIEF in Opposition re <u>455</u> MOTION in Limine <i>No. 6 to Exclude References to Plaintiffs' Motion for a Preliminary Injunction and Certain Related Materials</i> filed by Genentech, Inc..Reply Brief due date per Local Rules is 12/27/2021. (Balick, Steven) (Entered: 12/16/2021)
12/16/2021	<u>497</u>	[SEALED] ANSWERING BRIEF in Opposition re <u>457</u> MOTION in Limine <i>No. 7 to Preclude Genentech from Relying on the Court's Claim Construction Order to Refute Willfulness</i> filed by Genentech, Inc..Reply Brief due date per Local Rules is 12/27/2021. (Balick, Steven) (Entered: 12/16/2021)
12/16/2021	<u>498</u>	[SEALED] ANSWERING BRIEF in Opposition re <u>448</u> MOTION to Preclude ( <i>Genentech, Inc.'s Daubert Motion to Exclude Certain Opinions of Baxaltas Expert Leonard Paul Freedman</i> ) filed by Baxalta GmbH, Baxalta Incorporated.Reply Brief due date per Local Rules is 12/27/2021. (Attachments: # <u>1</u> Exhibit 1, # <u>2</u> Exhibit 2, # <u>3</u> Exhibit 3, # <u>4</u> Certificate of Service)(Dudash, Amy) (Entered: 12/16/2021)
12/16/2021	<u>499</u>	[SEALED] ANSWERING BRIEF in Opposition re <u>458</u> MOTION to Exclude Certain Opinions and Testimony of Drs. Steven Schwartz and Guy Young filed by Genentech, Inc..Reply Brief due date per Local Rules is 12/27/2021. (Balick, Steven) (Entered: 12/16/2021)
12/16/2021	<u>500</u>	[SEALED] ANSWERING BRIEF in Opposition re <u>450</u> MOTION to Preclude ( <i>Genentech, Inc.'s Daubert Motion to Exclude the Opinions of Baxalta's Expert Witness Dr. Jeffrey J. Gray and Related Opinions of Baxalta's Expert Witness Dr. Wayne A. Marasco</i> ) filed by Baxalta GmbH, Baxalta Incorporated.Reply Brief due date per Local Rules is 12/27/2021. (Attachments: # <u>1</u> Exhibit 1, # <u>2</u> Certificate of Service)(Dudash, Amy) (Entered: 12/16/2021)
12/16/2021	<u>501</u>	[SEALED] ANSWERING BRIEF in Opposition re <u>459</u> MOTION to Exclude Certain Opinions and Testimony of Dr. Richard Manning filed by Genentech, Inc..Reply Brief due date per Local Rules is 12/27/2021. (Balick, Steven) (Entered: 12/16/2021)

12/16/2021	<u>502</u>	[SEALED] ANSWERING BRIEF in Opposition re <u>460</u> MOTION to Exclude Opinions and Testimony of Dr. Dougald M. Monroe, III filed by Genentech, Inc..Reply Brief due date per Local Rules is 12/27/2021. (Balick, Steven) (Entered: 12/16/2021)
12/16/2021	<u>503</u>	[SEALED] DECLARATION of <i>Naz Wehrli in Support of Genentech, Inc.'s Oppositions to Baxalta's Motions In Limine and Daubert Motions</i> by Genentech, Inc.. (Attachments: # <u>1</u> Exhibit 1–28)(Balick, Steven) (Entered: 12/16/2021)
12/16/2021	<u>504</u>	[SEALED] ANSWERING BRIEF in Opposition re <u>451</u> MOTION to Preclude ( <i>Genentech, Inc.'s Daubert Motion to Exclude Certain Opinions of Baxalta's Expert Witness Dr. Sriram Krishnaswamy</i> ) filed by Baxalta GmbH, Baxalta Incorporated.Reply Brief due date per Local Rules is 12/27/2021. (Attachments: # <u>1</u> Exhibit 1, # <u>2</u> Exhibit 2, # <u>3</u> Exhibit 3, # <u>4</u> Exhibit 4, # <u>5</u> Exhibit 5, # <u>6</u> Exhibit 6, # <u>7</u> Exhibit 7, # <u>8</u> Exhibit 8, # <u>9</u> Certificate of Service)(Dudash, Amy) (Entered: 12/16/2021)
12/16/2021	<u>505</u>	[SEALED] ANSWERING BRIEF in Opposition re <u>452</u> MOTION to Preclude ( <i>Genentech, Inc.'s Daubert Motion to Exclude Certain Opinions of Baxalta's Expert James E. Malackowski</i> ) filed by Baxalta GmbH, Baxalta Incorporated.Reply Brief due date per Local Rules is 12/27/2021. (Attachments: # <u>1</u> Exhibit 1, # <u>2</u> Exhibit 2, # <u>3</u> Certificate of Service)(Dudash, Amy) (Entered: 12/16/2021)
12/16/2021	<u>506</u>	[SEALED] ANSWERING BRIEF in Opposition re <u>453</u> MOTION to Preclude ( <i>Genentech, Inc.'s Daubert Motion to Exclude Certain Opinions of Baxalta's Expert Dr. Wayne A. Marasco</i> ) filed by Baxalta GmbH, Baxalta Incorporated.Reply Brief due date per Local Rules is 12/27/2021. (Attachments: # <u>1</u> Exhibit 1, # <u>2</u> Exhibit 2, # <u>3</u> Exhibit 3, # <u>4</u> Certificate of Service)(Dudash, Amy) (Entered: 12/16/2021)
12/23/2021	<u>507</u>	[SEALED] Joint MOTION to Postpone Jury Trial – filed by Baxalta GmbH, Baxalta Incorporated. (Attachments: # <u>1</u> Certificate of Service)(Dudash, Amy) (Entered: 12/23/2021)
12/23/2021	<u>508</u>	[SEALED] REPLY BRIEF re <u>441</u> MOTION in Limine ( <i>Genentech, Inc.'s Motion In Limine No. 1 Regarding Literal Infringement</i> ) filed by Genentech, Inc.. (Attachments: # <u>1</u> Exhibit A–B)(Mayo, Andrew) (Entered: 12/23/2021)
12/23/2021	<u>509</u>	[SEALED] REPLY BRIEF re <u>443</u> MOTION in Limine ( <i>Genentech, Inc.'s Motion In Limine No. 2 to Preclude Improper Evidence of Willfulness</i> ) filed by Genentech, Inc.. (Attachments: # <u>1</u> Exhibit A)(Mayo, Andrew) (Entered: 12/23/2021)
12/23/2021	<u>510</u>	[SEALED] REPLY BRIEF re <u>444</u> MOTION in Limine ( <i>Genentech, Inc.'s Motion In Limine No. 3 to Preclude Named Inventor Dr. Scheiflinger From Offering Expert Opinions</i> ) filed by Genentech, Inc.. (Attachments: # <u>1</u> Exhibit A)(Mayo, Andrew) (Entered: 12/23/2021)
12/23/2021	<u>511</u>	REPLY BRIEF re <u>445</u> MOTION in Limine ( <i>Genentech, Inc.'s Motion In Limine No. 4 to Preclude Baxalta From Offering Written–Description Testimony that Factor X is an Obvious Choice for a Second Antigen for a Bispecific Antibody</i> ) filed by Genentech, Inc.. (Mayo, Andrew) (Entered: 12/23/2021)
12/23/2021	<u>512</u>	[SEALED] REPLY BRIEF re <u>446</u> MOTION in Limine ( <i>Genentech, Inc.'s Motion In Limine No. 5 to Preclude Baxalta From Presenting Arguments and Evidence Regarding Certain Deposited Hybridomas</i> ) filed by Genentech, Inc.. (Attachments: # <u>1</u> Exhibit A–D)(Mayo, Andrew) (Entered: 12/23/2021)
12/23/2021	<u>513</u>	[SEALED] REPLY BRIEF re <u>447</u> MOTION in Limine ( <i>Genentech, Inc.'s Motion In Limine No. 6 to Preclude Baxalta From Offering Testimony Regarding scFv Derivatives</i> ) filed by Genentech, Inc.. (Attachments: # <u>1</u> Exhibit A–C)(Mayo, Andrew) (Entered: 12/23/2021)
12/23/2021	<u>514</u>	[SEALED] REPLY BRIEF re <u>448</u> MOTION to Preclude ( <i>Genentech, Inc.'s Daubert Motion to Exclude Certain Opinions of Baxaltas Expert Leonard Paul Freedman</i> ) filed by Genentech, Inc.. (Mayo, Andrew) (Entered: 12/23/2021)
12/23/2021	<u>515</u>	[SEALED] REPLY BRIEF re <u>450</u> MOTION to Preclude ( <i>Genentech, Inc.'s Daubert Motion to Exclude the Opinions of Baxalta's Expert Witness Dr. Jeffrey J. Gray and Related Opinions of Baxalta's Expert Witness Dr. Wayne A. Marasco</i> ) filed by Genentech, Inc.. (Mayo, Andrew) (Entered: 12/23/2021)
12/23/2021	<u>516</u>	[SEALED] REPLY BRIEF re <u>451</u> MOTION to Preclude ( <i>Genentech, Inc.'s Daubert Motion to Exclude Certain Opinions of Baxalta's Expert Witness Dr. Sriram Krishnaswamy</i> ) filed by Genentech, Inc.. (Attachments: # <u>1</u> Exhibit A–B)(Mayo, Andrew) (Entered: 12/23/2021)

12/23/2021	<u>517</u>	[SEALED] REPLY BRIEF re <u>452</u> MOTION to Preclude ( <i>Genentech, Inc.'s Daubert Motion to Exclude Certain Opinions of Baxalta's Expert James E. Malackowski</i> ) filed by Genentech, Inc.. (Mayo, Andrew) (Entered: 12/23/2021)
12/23/2021	<u>518</u>	[SEALED] REPLY BRIEF re <u>453</u> MOTION to Preclude ( <i>Genentech, Inc.'s Daubert Motion to Exclude Certain Opinions of Baxalta's Expert Dr. Wayne A. Marasco</i> ) filed by Genentech, Inc.. (Attachments: # <u>1</u> Exhibit A)(Mayo, Andrew) (Entered: 12/23/2021)
12/23/2021	<u>519</u>	[SEALED] REPLY BRIEF re <u>438</u> MOTION in Limine to Exclude Evidence of Post–Priority Development Efforts filed by Baxalta GmbH, Baxalta Incorporated. (Attachments: # <u>1</u> Certificate of Service)(Dudash, Amy) (Entered: 12/23/2021)
12/23/2021	<u>520</u>	[SEALED] REPLY BRIEF re <u>439</u> MOTION in Limine No. 2 to Exclude John P. Sheehan, M.D. and William R. Strohl Reliance on Confidential Documents for Written Description, Enablement and Indefiniteness filed by Baxalta GmbH, Baxalta Incorporated. (Attachments: # <u>1</u> Exhibit 1, # <u>2</u> Certificate of Service)(Dudash, Amy) (Entered: 12/23/2021)
12/23/2021	<u>521</u>	REPLY BRIEF re <u>440</u> MOTION in Limine No. 3 to Exclude Certain Evidence of Japanese Proceedings Concerning the Japanese Counterpart of the Patent–in–Suit filed by Baxalta GmbH, Baxalta Incorporated. (Dudash, Amy) (Entered: 12/23/2021)
12/23/2021	<u>522</u>	[SEALED] REPLY BRIEF re <u>442</u> MOTION in Limine No. 4 to Exclude Any Genentech, Chugai, and Roche Patents Relating to Hemlibra filed by Baxalta GmbH, Baxalta Incorporated. (Attachments: # <u>1</u> Certificate of Service)(Dudash, Amy) (Entered: 12/23/2021)
12/23/2021	<u>523</u>	[SEALED] REPLY BRIEF re <u>449</u> MOTION in Limine No. 5 to Preclude Genentech from Introducing Evidence or Testimony Relating to Antibodies Falling Outside the Scope of the Claims to Support Its Written Description Defense filed by Baxalta GmbH, Baxalta Incorporated. (Attachments: # <u>1</u> Certificate of Service)(Dudash, Amy) (Entered: 12/23/2021)
12/23/2021	<u>524</u>	[SEALED] REPLY BRIEF re <u>455</u> MOTION in Limine No. 6 to Exclude References to Plaintiffs' Motion for a Preliminary Injunction and Certain Related Materials filed by Baxalta GmbH, Baxalta Incorporated. (Attachments: # <u>1</u> Exhibit 1, # <u>2</u> Certificate of Service)(Dudash, Amy) (Entered: 12/23/2021)
12/23/2021	<u>525</u>	REPLY BRIEF re <u>457</u> MOTION in Limine No. 7 to Preclude Genentech from Relying on the Court's Claim Construction Order to Refute Willfulness filed by Baxalta GmbH, Baxalta Incorporated. (Dudash, Amy) (Entered: 12/23/2021)
12/23/2021	<u>526</u>	[SEALED] REPLY BRIEF re <u>458</u> MOTION to Exclude Certain Opinions and Testimony of Drs. Steven Schwartz and Guy Young filed by Baxalta GmbH, Baxalta Incorporated. (Attachments: # <u>1</u> Certificate of Service)(Dudash, Amy) (Entered: 12/23/2021)
12/23/2021	<u>527</u>	[SEALED] REPLY BRIEF re <u>459</u> MOTION to Exclude Certain Opinions and Testimony of Dr. Richard Manning filed by Baxalta GmbH, Baxalta Incorporated. (Attachments: # <u>1</u> Exhibit 1, # <u>2</u> Certificate of Service)(Dudash, Amy) (Entered: 12/23/2021)
12/23/2021	<u>528</u>	[SEALED] REPLY BRIEF re <u>460</u> MOTION to Exclude Opinions and Testimony of Dr. Dougald M. Monroe, III filed by Baxalta GmbH, Baxalta Incorporated. (Attachments: # <u>1</u> Exhibit A, # <u>2</u> Exhibit B, # <u>3</u> Exhibit C, # <u>4</u> Exhibit D, # <u>5</u> Certificate of Service)(Dudash, Amy) (Entered: 12/23/2021)
12/23/2021		ORAL ORDER granting <u>507</u> the parties Joint Motion to Postpone Trial. The Pretrial Conference scheduled for January 19, 2022, and the Trial scheduled for January 24, 2022, are hereby postponed. Ordered by Judge Timothy B. Dyk on 12/23/2021. (smg) (Entered: 12/27/2021)
12/27/2021	<u>529</u>	REDACTED VERSION of <u>489</u> Answering Brief in Opposition by Genentech, Inc.. (Mayo, Andrew) (Entered: 12/27/2021)
12/27/2021	<u>530</u>	REDACTED VERSION of <u>490</u> Answering Brief in Opposition, by Genentech, Inc.. (Mayo, Andrew) (Entered: 12/27/2021)
12/27/2021	<u>531</u>	REDACTED VERSION of <u>491</u> Answering Brief in Opposition, by Genentech, Inc.. (Mayo, Andrew) (Entered: 12/27/2021)
12/27/2021	<u>532</u>	REDACTED VERSION of <u>493</u> Answering Brief in Opposition, by Genentech, Inc.. (Mayo, Andrew) (Entered: 12/27/2021)

12/27/2021	<u>533</u>	REDACTED VERSION of <u>494</u> Answering Brief in Opposition, by Genentech, Inc.. (Mayo, Andrew) (Entered: 12/27/2021)
12/27/2021	<u>534</u>	REDACTED VERSION of <u>497</u> Answering Brief in Opposition, by Genentech, Inc.. (Mayo, Andrew) (Entered: 12/27/2021)
12/27/2021	<u>535</u>	REDACTED VERSION of <u>499</u> Answering Brief in Opposition, by Genentech, Inc.. (Mayo, Andrew) (Entered: 12/27/2021)
12/27/2021	<u>536</u>	REDACTED VERSION of <u>501</u> Answering Brief in Opposition by Genentech, Inc.. (Mayo, Andrew) (Entered: 12/27/2021)
12/27/2021	<u>537</u>	REDACTED VERSION of <u>502</u> Answering Brief in Opposition by Genentech, Inc.. (Mayo, Andrew) (Entered: 12/27/2021)
12/27/2021	<u>538</u>	REDACTED VERSION of <u>503</u> Declaration of <i>Naz Wehrli</i> by Genentech, Inc.. (Attachments: # <u>1</u> Exhibit 1–28)(Mayo, Andrew) (Entered: 12/27/2021)
12/27/2021	<u>539</u>	REDACTED VERSION of <u>485</u> Answering Brief in Opposition, by Baxalta GmbH, Baxalta Incorporated. (Attachments: # <u>1</u> Exhibit 1, # <u>2</u> Exhibit 2, # <u>3</u> Exhibit 3, # <u>4</u> Exhibit 4)(Dudash, Amy) (Entered: 12/27/2021)
12/27/2021	<u>540</u>	REDACTED VERSION of <u>486</u> Answering Brief in Opposition, by Baxalta GmbH, Baxalta Incorporated. (Attachments: # <u>1</u> Exhibit 1, # <u>2</u> Exhibit 2, # <u>3</u> Exhibit 3, # <u>4</u> Exhibit 4, # <u>5</u> Exhibit 5)(Dudash, Amy) (Entered: 12/27/2021)
12/27/2021	<u>541</u>	REDACTED VERSION of <u>487</u> Answering Brief in Opposition, by Baxalta GmbH, Baxalta Incorporated. (Dudash, Amy) (Entered: 12/27/2021)
12/27/2021	<u>542</u>	REDACTED VERSION of <u>488</u> Answering Brief in Opposition, by Baxalta GmbH, Baxalta Incorporated. (Dudash, Amy) (Entered: 12/27/2021)
12/27/2021	<u>543</u>	REDACTED VERSION of <u>492</u> Answering Brief in Opposition., by Baxalta GmbH, Baxalta Incorporated. (Attachments: # <u>1</u> Exhibit 1, # <u>2</u> Exhibit 2, # <u>3</u> Exhibit 3, # <u>4</u> Exhibit 4, # <u>5</u> Exhibit 5, # <u>6</u> Exhibit 6, # <u>7</u> Exhibit 7, # <u>8</u> Exhibit 8, # <u>9</u> Exhibit 9, # <u>10</u> Exhibit 10)(Dudash, Amy) (Entered: 12/27/2021)
12/27/2021	<u>544</u>	REDACTED VERSION of <u>495</u> Answering Brief in Opposition, by Baxalta GmbH, Baxalta Incorporated. (Attachments: # <u>1</u> Exhibit 1, # <u>2</u> Exhibit 2, # <u>3</u> Exhibit 3)(Dudash, Amy) (Entered: 12/27/2021)
12/27/2021	<u>545</u>	REDACTED VERSION of <u>498</u> Answering Brief in Opposition, by Baxalta GmbH, Baxalta Incorporated. (Attachments: # <u>1</u> Exhibit 1, # <u>2</u> Exhibit 2, # <u>3</u> Exhibit 3)(Dudash, Amy) (Entered: 12/27/2021)
12/27/2021	<u>546</u>	REDACTED VERSION of <u>500</u> Answering Brief in Opposition, by Baxalta GmbH, Baxalta Incorporated. (Attachments: # <u>1</u> Exhibit 1)(Dudash, Amy) (Entered: 12/27/2021)
12/27/2021	<u>547</u>	REDACTED VERSION of <u>504</u> Answering Brief in Opposition., by Baxalta GmbH, Baxalta Incorporated. (Attachments: # <u>1</u> Exhibit 1, # <u>2</u> Exhibit 2, # <u>3</u> Exhibit 3, # <u>4</u> Exhibit 4, # <u>5</u> Exhibit 5, # <u>6</u> Exhibit 6, # <u>7</u> Exhibit 7, # <u>8</u> Exhibit 8)(Dudash, Amy) (Entered: 12/27/2021)
12/27/2021	<u>548</u>	REDACTED VERSION of <u>505</u> Answering Brief in Opposition, by Baxalta GmbH, Baxalta Incorporated. (Attachments: # <u>1</u> Exhibit 1, # <u>2</u> Exhibit 2)(Dudash, Amy) (Entered: 12/27/2021)
12/27/2021	<u>549</u>	REDACTED VERSION of <u>506</u> Answering Brief in Opposition, by Baxalta GmbH, Baxalta Incorporated. (Attachments: # <u>1</u> Exhibit 1, # <u>2</u> Exhibit 2, # <u>3</u> Exhibit 3)(Dudash, Amy) (Entered: 12/27/2021)
01/03/2022	<u>550</u>	REDACTED VERSION of <u>508</u> Reply Brief by Genentech, Inc.. (Mayo, Andrew) (Entered: 01/03/2022)
01/03/2022	<u>551</u>	REDACTED VERSION of <u>509</u> Reply Brief by Genentech, Inc.. (Mayo, Andrew) (Entered: 01/03/2022)
01/03/2022	<u>552</u>	REDACTED VERSION of <u>510</u> Reply Brief, by Genentech, Inc.. (Mayo, Andrew) (Entered: 01/03/2022)
01/03/2022	<u>553</u>	REDACTED VERSION of <u>512</u> Reply Brief, by Genentech, Inc.. (Mayo, Andrew) (Entered: 01/03/2022)

01/03/2022	<u>554</u>	REDACTED VERSION of <u>513</u> Reply Brief, by Genentech, Inc.. (Mayo, Andrew) (Entered: 01/03/2022)
01/03/2022	<u>555</u>	REDACTED VERSION of <u>514</u> Reply Brief by Genentech, Inc.. (Mayo, Andrew) (Entered: 01/03/2022)
01/03/2022	<u>556</u>	REDACTED VERSION of <u>515</u> Reply Brief, by Genentech, Inc.. (Mayo, Andrew) (Entered: 01/03/2022)
01/03/2022	<u>557</u>	REDACTED VERSION of <u>516</u> Reply Brief, by Genentech, Inc.. (Mayo, Andrew) (Entered: 01/03/2022)
01/03/2022	<u>558</u>	REDACTED VERSION of <u>517</u> Reply Brief by Genentech, Inc.. (Mayo, Andrew) (Entered: 01/03/2022)
01/03/2022	<u>559</u>	REDACTED VERSION of <u>518</u> Reply Brief, by Genentech, Inc.. (Mayo, Andrew) (Entered: 01/03/2022)
01/03/2022	<u>560</u>	[SEALED] MEMORANDUM in Support re <u>519</u> Reply Brief [ <i>CORRECTED D.I. 519, Reply in Support of Baxalta's Motion in Limine No. 1 to Exclude Evidence of Post-Priority Date Development Efforts</i> ] filed by Baxalta GmbH, Baxalta Incorporated. Answering Brief/Response due date per Local Rules is 1/18/2022. (Attachments: # <u>1</u> Exhibit A, # <u>2</u> Certificate of Service)(Dudash, Amy) (Entered: 01/03/2022)
01/03/2022	<u>561</u>	[SEALED] EXHIBIT re <u>528</u> Reply Brief, [ <i>Corrected Exhibit A to Baxalta's Reply in Support of Its Motion to Exclude Certain Opinions and Testimony of Dr. Dougald M. Monroe, III</i> ] by Baxalta GmbH, Baxalta Incorporated. (Attachments: # <u>1</u> Certificate of Service)(Dudash, Amy) (Entered: 01/03/2022)
01/03/2022	<u>562</u>	REQUEST for Oral Argument by Genentech, Inc. re <u>450</u> MOTION to Preclude ( <i>Genentech, Inc.'s Daubert Motion to Exclude the Opinions of Baxalta's Expert Witness Dr. Jeffrey J. Gray and Related Opinions of Baxalta's Expert Witness Dr. Wayne A. Marasco</i> ), <u>442</u> MOTION in Limine No. 4 to Exclude Any Genentech, Chugai, and Roche Patents Relating to Hemlibra, <u>446</u> MOTION in Limine ( <i>Genentech, Inc.'s Motion In Limine No. 5 to Preclude Baxalta From Presenting Arguments and Evidence Regarding Certain Deposited Hybridomas</i> ), <u>441</u> MOTION in Limine ( <i>Genentech, Inc.'s Motion In Limine No. 1 Regarding Literal Infringement</i> ), <u>440</u> MOTION in Limine No. 3 to Exclude Certain Evidence of Japanese Proceedings Concerning the Japanese Counterpart of the Patent-in-Suit, <u>451</u> MOTION to Preclude ( <i>Genentech, Inc.'s Daubert Motion to Exclude Certain Opinions of Baxalta's Expert Witness Dr. Sriram Krishnaswamy</i> ), <u>458</u> MOTION to Exclude Certain Opinions and Testimony of Drs. Steven Schwartz and Guy Young , <u>455</u> MOTION in Limine No. 6 to Exclude References to Plaintiffs' Motion for a Preliminary Injunction and Certain Related Materials, <u>447</u> MOTION in Limine ( <i>Genentech, Inc.'s Motion In Limine No. 6 to Preclude Baxalta From Offering Testimony Regarding scFv Derivatives</i> ), <u>445</u> MOTION in Limine ( <i>Genentech, Inc.'s Motion In Limine No. 4 to Preclude Baxalta From Offering Written-Description Testimony that Factor X is an Obvious Choice for a Second Antigen for a Bispecific Antibody</i> ), <u>443</u> MOTION in Limine ( <i>Genentech, Inc.'s Motion In Limine No. 2 to Preclude Improper Evidence of Willfulness</i> ), <u>448</u> MOTION to Preclude ( <i>Genentech, Inc.'s Daubert Motion to Exclude Certain Opinions of Baxaltas Expert Leonard Paul Freedman</i> ), <u>460</u> MOTION to Exclude Opinions and Testimony of Dr. Dougald M. Monroe, III , <u>444</u> MOTION in Limine ( <i>Genentech, Inc.'s Motion In Limine No. 3 to Preclude Named Inventor Dr. Scheiflinger From Offering Expert Opinions</i> ), <u>457</u> MOTION in Limine No. 7 to Preclude Genentech from Relying on the Court's Claim Construction Order to Refute Willfulness, <u>453</u> MOTION to Preclude ( <i>Genentech, Inc.'s Daubert Motion to Exclude Certain Opinions of Baxalta's Expert Dr. Wayne A. Marasco</i> ), <u>459</u> MOTION to Exclude Certain Opinions and Testimony of Dr. Richard Manning , <u>452</u> MOTION to Preclude ( <i>Genentech, Inc.'s Daubert Motion to Exclude Certain Opinions of Baxalta's Expert James E. Malackowski</i> ), <u>438</u> MOTION in Limine to Exclude Evidence of Post-Priority Development Efforts, <u>439</u> MOTION in Limine No. 2 to Exclude John P. Sheehan, M.D. and William R. Strohl Reliance on Confidential Documents for Written Description, Enablement and Indefiniteness, <u>449</u> MOTION in Limine No. 5 to Preclude Genentech from Introducing Evidence or Testimony Relating to Antibodies Falling Outside the Scope of the Claims to Support Its Written Description Defense. (Mayo, Andrew) (Entered: 01/03/2022)
01/04/2022	<u>563</u>	REDACTED VERSION of <u>520</u> Reply Brief, by Baxalta GmbH, Baxalta Incorporated. (Attachments: # <u>1</u> Exhibit 1)(Dudash, Amy) (Entered: 01/04/2022)

01/04/2022	<u>564</u>	REDACTED VERSION of <u>522</u> Reply Brief, by Baxalta GmbH, Baxalta Incorporated. (Dudash, Amy) (Entered: 01/04/2022)
01/04/2022	<u>565</u>	REDACTED VERSION of <u>523</u> Reply Brief, by Baxalta GmbH, Baxalta Incorporated. (Dudash, Amy) (Entered: 01/04/2022)
01/04/2022	<u>566</u>	REDACTED VERSION of <u>524</u> Reply Brief, by Baxalta GmbH, Baxalta Incorporated. (Attachments: # <u>1</u> Exhibit 1)(Dudash, Amy) (Entered: 01/04/2022)
01/04/2022	<u>567</u>	REDACTED VERSION of <u>526</u> Reply Brief by Baxalta GmbH, Baxalta Incorporated. (Dudash, Amy) (Entered: 01/04/2022)
01/04/2022	<u>568</u>	REDACTED VERSION of <u>527</u> Reply Brief by Baxalta GmbH, Baxalta Incorporated. (Attachments: # <u>1</u> Exhibit 1)(Dudash, Amy) (Entered: 01/04/2022)
01/04/2022	<u>569</u>	REDACTED VERSION of <u>528</u> Reply Brief, by Baxalta GmbH, Baxalta Incorporated. (Attachments: # <u>1</u> Exhibit A, # <u>2</u> Exhibit B, # <u>3</u> Exhibit C, # <u>4</u> Exhibit D)(Dudash, Amy) (Entered: 01/04/2022)
01/04/2022	<u>570</u>	REDACTED VERSION of <u>560</u> MEMORANDUM in Support, by Baxalta GmbH, Baxalta Incorporated. (Attachments: # <u>1</u> Exhibit A)(Dudash, Amy) (Entered: 01/04/2022)
01/05/2022	<u>571</u>	ORDER denying <u>435</u> Motion to Seal. (See Order for further details). Signed by Judge Timothy Belcher Dyk on 01/05/2022. (smg) (Entered: 01/06/2022)
01/12/2022	<u>572</u>	NOTICE requesting Clerk to remove Alexander F. Atkins as co-counsel.. (Mayo, Andrew) (Entered: 01/12/2022)
01/13/2022	<u>573</u>	[UNSEALED] MEMORANDUM OPINION. Signed by Judge Timothy Belcher Dyk on 1/13/2022.This order has been emailed to local counsel. (myr) (Entered: 01/13/2022)
01/13/2022	<u>574</u>	ORDER granting <u>407</u> Genentech Inc.'s Motion for Summary Judgment. See the accompanying Memorandum Opinion for additional details. The parties shall meet and confer and propose any redactions to the Memorandum Opinion on or before January 18, 2022. Signed by Judge Timothy Belcher Dyk on 1/13/2022. (myr) (Entered: 01/13/2022)
01/13/2022	<u>575</u>	ORAL ORDER. In light of the court's <u>574</u> Order granting Genentech Inc.'s Motion for Summary Judgment, the parties' outstanding pre-trial motions, ECF Nos. 438-453, 455, 457-460, are hereby denied as moot. Entered by Judge Timothy Belcher Dyk on 1/13/2022. (nmg) (Entered: 01/13/2022)
01/18/2022	<u>576</u>	Letter to The Honorable Timothy B. Dyk from Steven J. Balick regarding the Parties' Joint Position Concerning Redaction of the Court's January 13, 2022 Memorandum Opinion (D.I. 573). (Balick, Steven) (Entered: 01/18/2022)
01/19/2022	<u>577</u>	ORAL ORDER: In light of <u>576</u> the parties' January 18, 2022, Letter informing the court that they have not requested redactions to the January 13, 2022, Memorandum Opinion, the clerk is hereby directed to unseal the Memorandum Opinion <u>573</u> in its entirety. Signed by Judge Timothy Belcher Dyk on 1/19/2022. (myr) (Entered: 01/19/2022)
01/19/2022		Remark: D.I. <u>573</u> Memorandum Opinion UNSEALED per Oral Order D.I. 577 (myr) (Entered: 01/19/2022)
02/08/2022	<u>578</u>	NOTICE OF APPEAL to the Federal Circuit of <u>574</u> Order on Motion for Summary Judgment, <u>573</u> Memorandum Opinion . Appeal filed by Baxalta GmbH, Baxalta Incorporated. (Dudash, Amy) (Entered: 02/08/2022)
02/08/2022		APPEAL – Credit Card Payment of \$505.00 received re <u>578</u> Notice of Appeal (Federal Circuit) filed by Baxalta GmbH, Baxalta Incorporated. ( Filing fee \$505, receipt number ADEDC-3798835.) (Dudash, Amy) (Entered: 02/08/2022)
02/09/2022		Notice of Appeal and Docket Sheet to US Court of Appeals for the Federal Circuit re <u>578</u> Notice of Appeal (Federal Circuit). (srs) (Entered: 02/09/2022)
02/10/2022	<u>579</u>	NOTICE of Docketing Record on Appeal from USCA for the Federal Circuit re <u>578</u> Notice of Appeal (Federal Circuit) filed by Baxalta GmbH and Baxalta Incorporated. USCA Case Number 22-1461. (nmg) (Entered: 02/10/2022)
02/24/2022	<u>580</u>	TRANSCRIPT REQUEST by Baxalta GmbH, Baxalta Incorporated TRANSCRIPT IS ALREADY ON FILE (Dudash, Amy) (Entered: 02/24/2022)

03/31/2022		CASE NO LONGER REFERRED to Chief Magistrate Judge Thyng for the purpose of exploring ADR. Pursuant to the Court's <u>Standing Order No. 2022-2</u> , dated March 14, 2022, "[u]nless otherwise directed by the Court, Magistrate Judges will no longer engage in alternative dispute resolution of patent and securities cases." <i>See also</i> 28 U.S.C. § 652(b). (Taylor, Daniel) (Entered: 03/31/2022)
------------	--	---



**IN THE UNITED STATES DISTRICT COURT  
FOR THE DISTRICT OF DELAWARE**

BAXALTA INCORPORATED, a Delaware  
corporation; and BAXALTA GMBH, a Swiss  
company,

Plaintiffs,

v.

GENENTECH, INC., a Delaware corporation; and  
CHUGAI PHARMACEUTICAL CO., LTD., a  
Japanese company,

Defendants.

C.A. No. \_\_\_\_\_

JURY TRIAL DEMANDED

**COMPLAINT FOR PATENT INFRINGEMENT AND  
DECLARATORY JUDGMENT OF PATENT INFRINGEMENT**

Baxalta Incorporated and Baxalta GmbH (collectively, “Baxalta”), by its attorneys, alleges as follows for its Complaint for Patent Infringement and Declaratory Judgment of Patent Infringement against Genentech, Inc. (“Genentech”) and Chugai Pharmaceutical Co., Ltd. (“Chugai”) (collectively, “Defendants”):

**NATURE OF THE ACTION**

1. This is an action for patent infringement and for a declaratory judgment of patent infringement of United States Patent No. 7,033,590. This action arises out of Defendants’ current and/or imminent manufacture, use, sale, offer to sell within the United States, and/or importation into the United States of Defendants’ humanized bispecific antibody that binds Factor IX/IXa and Factor X to treat hemophilia A. Defendants developed this antibody under the name “emicizumab.” Emicizumab is also known as ACE910.

billion (US \$2.413 billion) annually from 2012 and cost CHF 2.7 billion (US \$2.715 billion).

In March 2010, Roche planned to strengthen its position in oncology and expand its metabolism, inflammation and central nervous system disease therapeutic areas.

In February 2007, Roche introduced a new operating model for its global R&D activities, organized around Disease Biology Areas (DBAs). Roche expected to increase its R&D staff numbers and \$4.8 billion budget in 2007. The DBAs would be divided into oncology, virology, inflammation, metabolism and central nervous system, with leadership teams located in Basel, Switzerland, Nutley, NJ and Palo Alto, CA. The teams would manage compounds from drug discovery through to medical proof-of-concept, and to the market. Therapeutic protein research would also be stepped up at the company's Penzberg, Germany site, and R&D activities at its site in Shanghai, China, would be expanded. This new model was implemented in July 2007.

In October 2002, Roche acquired a majority ownership of Chugai Pharmaceutical following Chugai's merger with Roche's subsidiary, Nippon Roche, with Chugai as the surviving company.

In September 1990, Roche acquired a majority interest in Genentech.

#### COMPANY LOCATION

In November 2015, the company planned to exit four manufacturing sites in Clarecastle, Ireland; Leganes, Spain; Segrate, Italy; and Florence, US, to restructure its manufacturing network for small molecules which are produced in lower volumes than traditional medicines and require novel manufacturing technologies. In an effort to minimise job reductions, the company was actively looking into divestment opportunities for these facilities. Also, Roche planned to invest CHF 300 million (US \$298.510 million) to strengthen development and launch capabilities for specialised medicines at Kaiseraugst, Switzerland.

In October 2014, Roche planned to invest CHF 3 billion (US \$3.17 billion) over the next 10 years in its Basel site for the construction of a new R&D centre which was scheduled for completion by end of 2018, a new office building which was expected to be ready for occupation by 2021, in upgrading the infrastructure and to renovate the historic Salvisberg building.

In December 2012, Roche planned to invest over CHF 240 million (US \$258 million) in its Penzberg biotechnology center near Munich, Germany, to construct a new three-structured, 26,000-square metered production facility, to be known as Diagnostics Operations Complex II (DOCII). The facility was scheduled to be operational in December 2014.

In September 2012, Roche planned to establish a Translational Clinical Research Center (TCRC) at the Alexandria Center for Life Science in New York, NY. Roche agreed an 11-year lease and was to relocate its TCRC team to the facility in the fourth quarter of 2013. In October 2013, the TCRC was opened, with 250 staff are to move in on January 02, 2014.

In July 2012, it was announced that Roche would employ an additional 1000 people in China during the following year.

In September 2011, Roche opened a clinical supply manufacturing facility at its site in Nutley, NJ.

In August 2011, the company's subsidiary, Roche Canada, was to invest CAD \$190 million (US \$193.3 million) over five years in a global development site in Mississauga, Ontario, Canada.

In June 2011, Roche launched a new R&D Institute in France focused on collaborative translational research and medicine.

In May 2009, Roche opened a Joint Science & Technology Laboratory in Basel, Switzerland, to investigate in vitro hepatic and cardiac toxicities in vitro using Roche Diagnostics' cell analysis technology.

In January 2009, the company began construction of a new R&D building in Basel, Switzerland, which would cost CHF 250 million (US \$219 million). The facility would conduct work on solid and liquid dosage forms for new active substances and supply investigational drugs for global clinical trials.

In November 2008, Roche planned to investigate alternatives to its planned high-rise development in Basel, Switzerland.

In April 2007, Roche planned to build a new \$60 million production unit in order to expand its Florence, CA manufacturing facility. Construction would start in mid-2007 with completion by the end of 2008.

In October 2001, Roche dedicated a new research center for its pharmaceuticals and diagnostics divisions in Penzberg, Germany. The new buildings provided space for 360 employees and represented an investment by Roche of over 140 million Deutschmarks.

By June 2001, Roche had announced its plans to discontinue all research activities at its UK site in Welwyn.

[Return to Table of Contents](#)



serve as a centre for the discovery of innovative biotherapeutics. Roche planned to use ARIUS' FunctionFIRST antibody technology to strengthen its development pipeline, initially within the areas of oncology and inflammatory diseases. The deal was expected to close in the third quarter of 2008. In September 2008, RiskMetrics ISS Canada, an independent proxy firm, stated they had no objection to the transaction and recommended that ARIUS Research shareholders vote for the acquisition. In September 2008, ARIUS' shareholders approved the acquisition. At that time, the deal was to close 'shortly'. Later in September 2008, Roche completed its acquisition of ARIUS.

Also in July 2008, Roche entered into a definitive agreement for the acquisition of Mirus Bio for \$125 million. Roche would gain access to several nucleic acid-based technologies from Mirus, including its RNAi delivery platform. Roche planned to maintain an RNAi research center at Mirus' site in Madison, WI. Mirus' transfection reagents business would be divested into a standalone business to be known as Mirus Bio LLC. The deal was expected to close in the second half of 2008.

Earlier in July 2008, Roche planned to acquire all of Genentech's outstanding shares priced at \$89 each for \$43.7 billion. Genentech would merge with a Roche subsidiary and operate as an independent R&D center within Roche in South San Francisco, CA. Roche's Palo Alto Virology R&D activities and Pharma commercial operations would relocate to South San Francisco. In addition, Roche's Palo Alto Inflammation group would become part of its Nutley, NJ, R&D organization. The merger was subject to Genentech shareholder approval. Later in July 2008, Genentech announced that it had formed a special committee of its Board to assess Roche's proposal. In August 2008, the shareholders of Genentech objected to Roche's proposal, claiming it was 'unfair and inappropriate'. Later that month, Genentech's special committee concluded that Roche's offer undervalued the company. In January 2009, Roche planned to start a cash tender offer for all of Genentech's stock, priced at \$86.50 per share, within 2 weeks. The offer is conditional upon the majority of shares being tendered, and Roche obtaining sufficient funds to finance the deal. Approval of Genentech's Board or special committee was not required. In February 2009, Roche began its tender offer for Genentech. The offer was scheduled to expire on March 12, 2009, unless extended. At that time, Genentech advised its shareholders to take no action and planned to issue its formal position on the offer within 10 days [982828]. Later that month, Genentech advised its shareholders to reject Roche's offer as it was inadequate. In March 2009, Roche offered \$93 per share and extended the offer until March 20, 2009. By that time, 0.5 million shares had been tendered. However, Genentech advised its shareholders to take no action. It intended to take a formal position on the offer 'promptly'. Later in March 2009, Roche and Genentech agreed to a \$95 per share deal worth \$46.8 billion. Roche would amend the existing tender offer to reflect the increased price; by that time a total of 2.9 million shares had been tendered. If the tender offer is completed, Roche would consummate a second-step merger in which all remaining public shareholders would receive \$95 per share for their shares. Later that month, Roche completed the tender offer with 395.7 million shares (84.7%) tendered. A further 3% of the stock had been guaranteed to be delivered within the next 3 days. This would bring Roche's stake in Genentech to 96.2%. The merger completed later that month. Genentech became a wholly-owned subsidiary of Roche and its stock was removed from the NYSE.

In September 1990, Roche acquired a majority interest (60%) in Genentech. The collaboration between the companies was strengthened in July 1995 with an agreement to extend Roche's option to purchase the outstanding redeemable common stock of Genentech. In June 1999, Roche acquired the remaining outstanding shares of Genentech for \$3.8 billion. In October 1999, Genentech reported the offering of 20 million shares of its common stock in a public offering by Roche Holdings. The underwriters of the offering had an option to purchase an additional two million shares of Genentech common stock to cover over-allotments. As a result of the offering, Roche's ownership of Genentech was to be reduced to approximately 66 percent. In March 2000, Genentech offered 17.3 million shares of its common stock in public offering by Roche Holdings. The Genentech common stock was being offered to the public at a price of \$163 per share. The underwriters of the offering had an option to purchase an additional 1.7 million shares to cover over allotments. This offer would reduce Roche's ownership of Genentech to approximately 58% if the over allotment option was exercised, or 59% if it was not.

In April 2008, Roche Holding agreed to acquire Piramed in a \$160 million cash deal. Roche would purchase 100% of Piramed's shares and would gain rights to the company's oncology program. Piramed would receive a \$15 million milestone payment upon initiation of phase II trials. The transaction was expected to close in the second quarter of 2008. In May 2008, the acquisition was completed.

In February 2008, Roche completed its tender offer for 93.7% of Ventana Medical System's outstanding shares. In June 2007, Roche offered to buy Ventana Medical Systems for \$3 billion in a cash tender offer. However, Ventana's Board refused to enter discussions with the company and advised its shareholders not to act on the offer until it had considered it. Roche planned to buy Ventana's outstanding shares for \$75 each, a 44% premium on its closing price on June 22, 2007 and a 55% premium to its 3-month average. Ventana would become a part of Roche Diagnostics. In September 2007, Roche extended its offer to acquire all of the outstanding common shares of Ventana at \$75 per share in cash to November 01, 2007. The tender offer was previously due to expire on September 19, 2007. Ventana's Board recommended that stockholders not tender any of their shares. This followed earlier attempts in July and August 2007 by Roche to acquire Ventana's outstanding shares and the subsequent recommendation by the Ventana Board that stockholders reject the offer. In October 2007, Roche extended its offer to acquire all of the outstanding common shares of Ventana to January 17, 2008, other terms and conditions of the offer remained unchanged. At this time, 99.5% of Ventana's investors had turned down Roche's offer. In November 2007, the companies entered into a confidentiality agreement, which allowed Roche to commence due diligence and have appropriate access to non-public information regarding Ventana. There was no assurance that a definitive agreement would be reached. In January 2008, Roche extended its unsolicited tender offer for the fifth time, to March 14, 2008. By January 16, 2008, approximately 62,401

[Return to Table of Contents](#)



**IN THE UNITED STATES DISTRICT COURT  
FOR THE DISTRICT OF DELAWARE**

BAXALTA INCORPORATED, a Delaware corporation; and BAXALTA GMBH, a Swiss company,

Plaintiffs,

v.

GENENTECH, INC., a Delaware corporation; and  
CHUGAI PHARMACEUTICAL CO., LTD., a Japanese company,

Defendants.

C.A. No. 17-509-GMS

JURY TRIAL DEMANDED

ORAL ARGUMENT REQUESTED

**BAXALTA'S MOTION FOR A PRELIMINARY INJUNCTION**

Colm F. Connolly (#3151)  
Amy M. Dudash (#5741)  
**MORGAN, LEWIS & BOCKIUS LLP**  
1007 North Orange Street, Suite 501  
Wilmington, DE 19801  
Telephone: (302) 574-3000  
cconnolly@morganlewis.com  
amy.dudash@morganlewis.com

Margaret A. McGreal  
Jessica A. Stow  
**MORGAN, LEWIS & BOCKIUS LLP**  
1701 Market Street  
Philadelphia, PA 19103  
Telephone: (215) 963-5000  
margaret.mcgregal@morganlewis.com  
jessica.stow@morganlewis.com

Michael J. Abernathy  
Christopher J. Betti  
Amanda S. Williamson  
Jennifer M. Dienes  
Maria E. Doukas  
**MORGAN, LEWIS & BOCKIUS LLP**  
77 West Wacker Drive, Fifth Floor  
Chicago, IL 60601  
Telephone: (312) 324-1000  
mike.abernathy@morganlewis.com  
christopher.betti@morganlewis.com  
amanda.williamson@morganlewis.com  
jennifer.dienes@morganlewis.com  
maria.doukas@morganlewis.com

*Attorneys for Plaintiffs*

Pursuant to Fed. R. Civ. P. 65, Baxalta Incorporated and Baxalta GmbH (“Plaintiffs”) hereby move for a preliminary injunction. The reasons for Plaintiffs’ Motion are set forth in the Memorandum of Law being filed contemporaneously herewith, which Plaintiffs incorporate herein by reference.

Date: December 14, 2017

/s/ Colm F. Connolly

Colm F. Connolly (#3151)

Amy M. Dudash (#5741)

**MORGAN, LEWIS & BOCKIUS LLP**

1007 North Orange Street, Suite 501

Wilmington, DE 19801

Telephone: (302) 574-3000

cconnolly@morganlewis.com

Michael J. Abernathy

Christopher J. Betti

Amanda S. Williamson

Jennifer M. Dienes

Maria E. Doukas

**MORGAN, LEWIS & BOCKIUS LLP**

77 West Wacker Drive, Fifth Floor

Chicago, IL 60601

Telephone: (312) 324-1000

mike.abernathy@morganlewis.com

christopher.betti@morganlewis.com

amanda.williamson@morganlewis.com

jennifer.dienes@morganlewis.com

maria.doukas@morganlewis.com

Margaret A. McGreal

Jessica A. Stow

**MORGAN, LEWIS & BOCKIUS LLP**

1701 Market Street

Philadelphia, PA 19103

Telephone: (215) 963-5000

margaret.mcgregal@morganlewis.com

jessica.stow@morganlewis.com

**IN THE UNITED STATES DISTRICT COURT  
FOR THE DISTRICT OF DELAWARE**

BAXALTA INCORPORATED, a Delaware corporation; and BAXALTA GMBH, a Swiss company,

Plaintiffs,

v.

GENENTECH, INC., a Delaware corporation; and  
CHUGAI PHARMACEUTICAL CO., LTD., a  
Japanese company,

Defendants.

C.A. No. 17-509-GMS

**DECLARATION OF SRIRAM KRISHNASWAMY, PH.D.  
IN SUPPORT OF PLAINTIFFS' MEMORANDUM IN  
SUPPORT OF THEIR MOTION FOR PRELIMINARY INJUNCTION**

I, Sriram Krishnaswamy, Ph.D., declare as follows:

**I. INTRODUCTION**

1. I make this Declaration at the request of Baxalta Incorporated and Baxalta GMBH (collectively, "Baxalta") in support of Plaintiffs' Motion for Preliminary Injunction.

2. My educational background, qualifications, and professional experience are summarized in ¶¶ 7 to 20 below, and are described more fully in my curriculum vitae which is attached hereto as **Exhibit A**.

3. In this Declaration, I provide my opinions regarding the infringement of claims 1, 2, 3, 4, 15, 19, and 20 ("Asserted Claims") of U.S. Patent No. 7,033,590 ("the '590 Patent"), which is attached hereto as **Exhibit B**, by Genentech, Inc.'s ("Genentech's") emicizumab, also known as ACE910 and sold under the tradename HEMLIBRA®. I am also providing my

opinions concerning the validity of the Asserted Claims of the '590 Patent, specifically, lack of anticipation, non-obviousness, and definiteness.

4. I am being compensated for my work in this litigation at my standard consulting rate of \$1,000 an hour. My compensation does not depend in any way on the opinions that I express in this Declaration or on the outcome of this litigation.

5. In reaching the opinions in this Declaration, I have considered, among other things, the materials identified in the body of this Declaration and in the List of Materials Reviewed attached hereto as **Exhibit C**. These materials include the '590 Patent, publications about ACE910, and the prior art reference identified in a patent infringement litigation concerning the Japanese counterpart to the '590 Patent now pending in the Tokyo District Court in Japan, Case No. Heisi 28 (Wa) 11475, between Baxalta and Chugai (the "Japanese Litigation"). Additionally, I have relied upon my professional and academic expertise in the fields of blood coagulation biochemistry, thrombosis, and hemostasis.

6. All of the opinions stated in this Declaration are based on my own personal knowledge and professional judgment; if called as a witness in this matter, I am prepared to testify competently about them.

## **II. QUALIFICATIONS**

7. As I noted above, a full listing of my qualifications as a technical expert for this litigation are detailed in my curriculum vitae, which is attached hereto as **Exhibit A**.

8. I have a Bachelor of Science degree in biology (1979) and a Ph.D. in biochemistry (1984), both from Syracuse University. My undergraduate and graduate research dealt with the enzymology of amino acid metabolism. In 1984, I received the Alexander Gourevitch Award for Excellence in Graduate Research from Syracuse University.

9. After receiving my Ph.D., I was a postdoctoral fellow (“postdoc”) in the Department of Biochemistry at the University of Vermont (1984-1987). My postdoctoral training was done in the laboratory of Kenneth Mann, which at the time was one of the premier laboratories in the world investigating the biochemistry of blood coagulation. During my postdoctoral training, I studied the enzymologic mechanisms involved in the reactions of blood coagulation, including the activation of human prothrombin to thrombin, which is essential to blood clot formation.

10. After obtaining the first FIRST grant awarded by the NHLBI, I was promoted to Research Assistant Professor (1987-1989) in the same department that I worked in as a postdoc. As a Research Assistant Professor, I continued my work studying the proteins and mechanisms involved in blood coagulation. I used purified proteins in reconstituted systems to draw mechanistic conclusions regarding the proteolytic activation steps in the coagulation cascade. I also employed biophysical and spectroscopic approaches to study the protein-protein and protein-membrane interactions that lead to enzyme complex and function. I expanded my approaches to include rapid kinetic measurements of these processes on the millisecond time scale. These and related strategies have formed the basis of my research throughout the rest of my career. Further, in 1989 I received the Brinkhous Young Investigator Prize in Thrombosis from the American Heart Association.

11. I was promoted to Assistant Professor on the tenure track in 1989 and held that position at the University of Vermont for one year. During that time, I continued my work with blood coagulation. I also taught courses on Enzymology and Physical Biochemistry.

12. I next took a position as an Assistant Professor in the Department of Medicine at Emory University (1990-1997). While an Assistant Professor, I continued my coagulation



research and taught the Medical Student Biochemistry class. I also lectured on Physical Biochemistry and Enzymology to graduate students and undergraduates.

13. I was promoted to Associate Professor of Medicine in 1997 (1997-1998).

14. In 1998, I left Emory University to become an Associate Professor (1998-2011) in the Department of Pediatrics and in the Department of Pharmacology at the University of Pennsylvania. I was also appointed as the Stokes Investigator at the Children's Hospital of Philadelphia Research Institute.

15. In 2011, I was promoted to Tenured Professor in the Department of Pediatrics and Professor (Secondary) in the Department of Pharmacology. At the University of Pennsylvania, I have continued my research in blood coagulation. I have expanded my repertoire of approaches to include other biophysical approaches, such as calorimetry and analytical ultracentrifugation. I have also used x-ray crystallography, small angle x-ray scattering, and single molecule cryo-EM. I have lectured in team-taught courses in Cardiovascular Pharmacology.

16. I continued in my role as Professor (Secondary) in the Department of Pharmacology until 2014, when I became a Professor (Secondary) in Systems Pharmacology and Translational Therapeutics and in the Department of Biochemistry and Biophysics as a member of the Structural Biology Group at the University of Pennsylvania.

17. I have continued my work at the University of Pennsylvania and currently hold the following positions: Professor in the Department of Pediatrics, Professor (Secondary) in Systems Pharmacology and Translational Therapeutics, and Professor (Secondary) in the Department of Biochemistry and Biophysics as a Member of the Structural Biology Group. Additionally, I have retained my appointment as Stokes Investigator. Throughout my academic

Case 1:17-cv-00509-LPS Document 48 Filed 12/14/17 Page 1 of 28 PageID #: 5241

**IN THE UNITED STATES DISTRICT COURT  
FOR THE DISTRICT OF DELAWARE**

BAXALTA INCORPORATED, a Delaware  
corporation; and BAXALTA GMBH, a Swiss  
company,

Plaintiffs,

v.

GENENTECH, INC., a Delaware corporation; and  
CHUGAI PHARMACEUTICAL CO., LTD., a  
Japanese company,

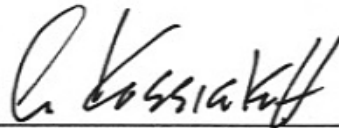
Defendants.

C.A. No. 17-509-GMS

**DECLARATION OF ANTHONY A. KOSSIAKOFF,  
PH.D, IN SUPPORT OF PLAINTIFFS' MEMORANDUM IN  
SUPPORT OF THEIR MOTION FOR PRELIMINARY INJUNCTION**

I declare under penalty of perjury under the laws of the United States that the foregoing is true and  
correct.

Date: December 13, 2017



Anthony Kossiakoff, Ph.D.

NOVEMBER/DECEMBER 1998  
Volume 9, Number 6

© Copyright 1998 by the American Chemical Society

# Bioconjugate Chemistry

## REVIEWS

---

### Bispecific Antibodies as Novel Bioconjugates

Y. Cao and M. R. Suresh\*

Faculty of Pharmacy and Pharmaceutical Sciences, University of Alberta,  
Edmonton, Alberta, Canada, T6G 2N8. Received April 30, 1998

---

Bispecific antibodies are unique macromolecular heterobifunctional cross-linkers with two different binding specificities within a single molecule. As ideal bioconjugates, they can specifically glue any two different molecules together without the need for chemical conjugation. With this unique feature, they have immense potential in biological and immunological fields. Their applications range from immunohistochemistry, immunoassays, radioimmunodiagnosis, radioimmunotherapy, and immunotherapy. Recently, a new second generation of bispecific molecules, bispecific single chain Fv and diabodies, has been produced by DNA recombinant technology. They can be considered as the ultimate magic bullets for in vivo applications. They may theoretically improve tumor or pathogen targeting and minimize side effects, eventually replacing the full-length bispecific antibodies. Emphasizing on developmental methodology and clinical applications of bispecific antibodies, this review gives a bird's-eye view of these unique bioconjugates.

---

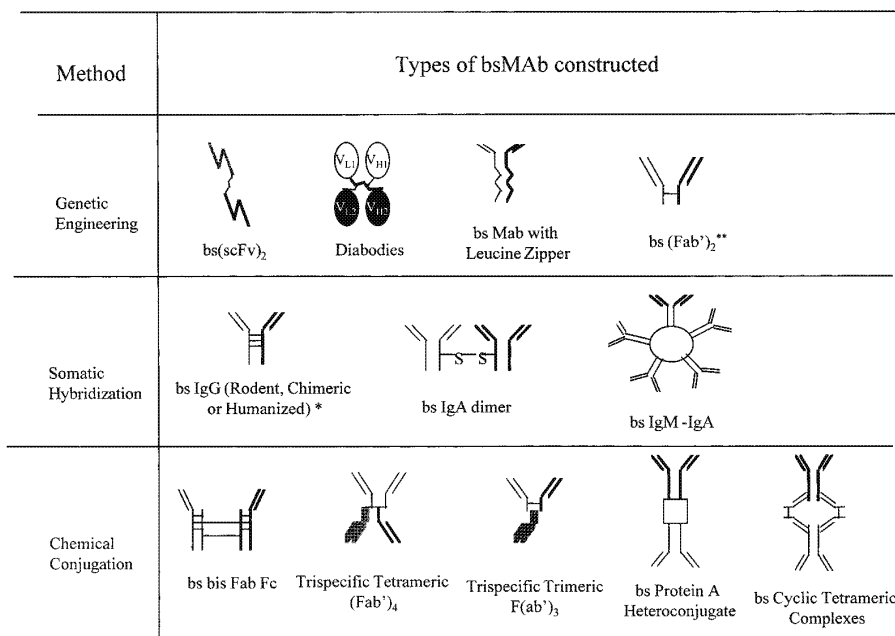
#### INTRODUCTION

Monospecific antibodies exemplified by the naturally occurring major IgG class have two identical antigen-binding paratopes due to their makeup with two identical heavy and light chains. On the contrary, bispecific antibodies are artificially engineered immunoglobulins with two distinct binding specificities. They were first generated 37 years ago by chemical methods (Nisonoff and Rivers, 1961). In the past two decades, hybridoma technology established itself as one of the cornerstones of modern biotechnology (Kohler and Milstein, 1975), and bispecific monoclonal antibody (bsMAb) represents second generation monoclonals developed for a variety of in vivo and in vitro uses.

A number of applications using monoclonal antibody (MAb) involve chemical manipulation of the antibody to create covalent immunoconjugates with radioisotopes, enzymes, toxins, drugs, and a variety of other haptens. Early chemical conjugation methods using homobifunctional cross-linkers generated complex aggregates of the two entities. Refinement in chemical conjugation methods, employing heterobifunctional cross-linkers, has mitigated the problems but not entirely eliminated them (Hermanson, 1996). BsMAb with intrinsic binding sites to any two antigens has the capability to form uniform, homogeneous, and reproducible immunoconjugates with one or two entities in a predetermined order (Suresh et al., 1986a). Since such binding is noncovalent and dependent on the affinity of the epitope-paratope interaction, bsMAb has a singular advantage to cross-link two cells for targeting in vivo (Staerz and Bevan, 1989). The

---

\* Author to whom correspondence should be addressed. Tel: 403-492-9233. Fax: 403-492-1217. E-mail: msuresh@pharmacy.ualberta.ca.



**Figure 1.** A schematic representation of a variety of bsMAbs. (\*) This has also been constructed by chemical and genetic methods. (\*\*) This has also been constructed by chemical methods.

latter feature has not been described to date using chemical conjugation for in vivo applications.

Current applications of bsMAB range from immuno-histochemistry (Milstein and Cuello, 1983), immunoassays (Kreutz and Suresh, 1995, 1997; Suresh et al., 1986a; Cao et al., 1998), complement-mediated cytotoxicity (Wong and Colvin, 1987), and in vivo retargeting (Fanger et al., 1995; Demanet et al., 1996; Somasundaram et al., 1996). BsMAB has great potential in a wide variety of research and eventually, clinical uses. Some of these exciting explorations include redirecting cytotoxicity to tumor cells, HIV virus, and infectious agents, targeting enzyme and site-specific activation of anticancer prodrugs and localizing fibrinogen activator to dissolve fibrin clots.

#### PRODUCTION OF BISPECIFIC ANTIBODIES

Many different types of bsMAB have been made (Figure 1) by one of the three general methods: (1) chemically cross-linking two antibody molecules or antibody fragments, (2) fusion of two different cell lines to form a quadroma or trioma, (3) recombinant DNA based approaches.

**1. Chemically Coupling.** More than 30 years ago, the first bispecific polyclonal antibodies were produced by chemically coupling two different polyclonal antibodies (Nisonoff and Rivers, 1961). This chemical manipulation involved the dissociation of the two different antibodies and reassociation of the two-half molecules. A portion of the reassembled antibodies was shown to be bispecific in nature by immunohistochemistry. With the advent of MAb and improvements in the chemistry of preferential formation of heteroconjugates, developing bispecific monoclonals became a reality eliminating the heterogeneous polyclonal reagents.

To prepare bsMAB, a large number of bifunctional reagents reactive with  $\epsilon$ -amino groups or hinge region thiol groups have been used. These cross-linkers can be classified into two categories, homo- and heterobifunc-

tional reagents. Homobifunctional reagents react with the free thiols generated upon reduction of inter heavy chain disulfide bonds. 5,5'-Dithiobis(2-nitrobenzoic acid) (DTNB) or *o*-phenylenedimaleimide (O-PDM) can activate thiol groups on Fab' fragments of MAb (Brenner et al., 1985; Glennie et al., 1987; Shalaby et al., 1992). DTNB acts to regenerate disulfide bonds between the two Fabs, whereas O-PDM acts to form a thioether bond between the two Fab'. Generally, the thioether bond of O-PDM could be more stable than the disulfide bond regenerated by DTNB (Glennie et al., 1987). In addition to dimeric bsMAbs, trimeric and tetrameric antibodies with two or three specificities have been constructed using O-PDM and shown to have more potent redirecting effector cell tumor cytotoxicity than corresponding dimeric antibodies (Tutt et al., 1991a,b).

Heterobifunctional reagents can introduce a reactive group onto a protein that will enable it to react with a second protein. *N*-Succinimidyl-3-(2-pyridyldithio) propionate (SPDP) has been used to react with primary amino groups to introduce free thiol groups (Segal et al., 1988; Jung et al., 1991). SPDP can combine any two proteins that have exposed amino groups including antibodies and Fab' fragments, regardless of class or isotype (Van Dijk et al., 1989). Unfortunately, this approach causes random cross-linking of the molecules and hence exhibits batch to batch variations.

The improvements in cross-linking technology has led at least one biotechnology company (Medarex Inc., NJ) to develop bispecific (Fab')<sub>2</sub> antibodies and evaluate their utility in FDA-approved human clinical trials. Chemical linking that has been highly refined is more straightforward and faster, produces higher yields, and the products are comparatively easier to purify (Glennie et al., 1987). However, denaturation of either protein or loss of antibody activity are concerns during these types of chemical reactions.

**2. Somatic Hybridization.** Somatic hybridization for generating bispecific antibodies first appeared in 1973

**CONFIDENTIAL MATERIAL OMITTED  
FROM THE FOLLOWING PAGES:**  
Appx6253, Appx6606

[illegible number]



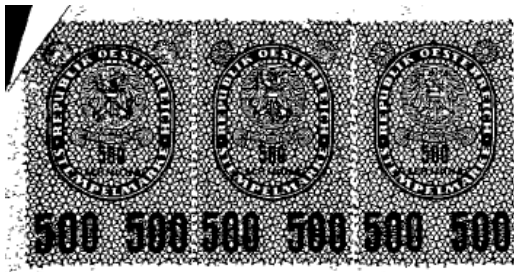
[handwritten entry – RE'D 03 NOV 2,000]

**AUSTRIAN PATENT OFFICE** [illegible entry WIPO/PCT.]

5

A-1014 VIENNA, KOHLMARKT 8 – 10

Handwritten entry - EP00/8936.]



Application number **A 1576/99**

[handwritten entry – E – JU.

[Republic of Austria revenue stamps]

The Austrian Patent Office confirms that

**The company BAXTER AKTIENGESELLSCHAFT**

10

**In A-1221 Vienna, Industriestrasse 67,**

submitted a patent application of **September 14, 1999** concerning

**“Antibodies and Antibody Derivatives Activating**

**Factor IX / Factor IXa”,**

and that the attached specification and drawings coincide with the original

15

specification and drawings submitted at the same time with this patent application.

Austrian Patent Office

Vienna, July 7, 2000

The president:



[Austrian Patent Office]

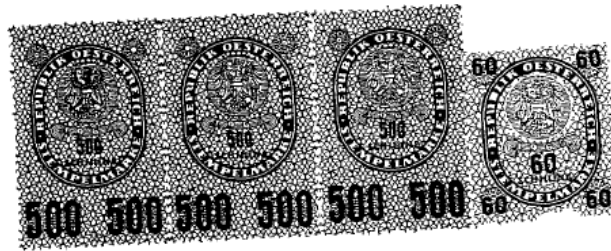
in behalf of



[Austrian Patent Office]

HRNCIR

Area upper inspector



[Republic of Austria revenue stamps]

AUSTRIAN PATENT OFFICE

Administration offices – Management

/1300 schillings 94.47 €

Office fee paid.

[sig.]

*Wegschaidt*



025791

**A1576 / 99 – 1**

(51) Int. Cl.:

R 35954

AT PATENT

(11) No. ORIGINAL TEXT

5 (73) Patent holder: BAXTER AKTIENGESELLSCHAFT

Vienna (AT)

10 (54) Subject matter: Antibodies and Antibody Derivatives Activating Factor IX /  
Factor IXa

(61) Addition to patent No.

(67) Conversion from utility model

(62) Division from:

15 (22) (21) Filed on: **14. SEP. 1999** [illegible – 1/4/Sept. 1999]

(33) (32) (31) Union priority:

(42) Beginning of patent term:

Longest possible term:

20 (45) Issued on:

(72) Inventor(s):

60) Dependence

025791

---

(56) References used for the evaluation of patentability:

5

Printed form PA 31. – Cover page of the specification – 5,000 – Zl. 3090/präs. 94

DVR: 0078018

10

[handwritten entry – 1/2.]

025791

The invention relates to factor IX / factor IXa antibodies and antibody derivatives.

Blood clots (thrombi) form by a series of zymogen activations. During the course of this enzymatic cascade the activated form of a factor catalyzes the activation of the next one. Thrombi are deposits of blood components on the surface of the vessel wall and consist for the most part of aggregations of blood platelets and insoluble, cross-linked fibrin. The formation of fibrin takes place by thrombin by limited proteolysis of fibrinogen. Thrombin is the end product of the clotting cascade, a course of zymogen activations on the surface of platelets, leukocytes and a plurality of vascular cells (K. G. Mann, Blood, 1990, vol. 76, pp. 1-16).

The activation of factor X by the complex consisting of the activated factor IX (fXa) and activated factor VIII (FVIIIa) constitutes a key step in the clotting. A lack of or a disturbed function of the components of this complex is associated with the blood clotting disturbance designated as hemophilia (J. E. Sadler & E.W. Davie: Hemophilia A, Hemophilia B, and von Willebrand's disease, in G. Stamatoyannopoulos et al. (eds) : The molecular basis of blood diseases. W.B. Saunders Co, Philadelphia, 1987, pp. 576-602). Hemophilia A designates a (functional) lack of the factor VIII activity, hemophilia B designates a lack of the factor IX activity.

The treatment of hemophilia A currently takes place by a replacement therapy by the administration of factor VIII concentrates, wherein in approximately 20-30% of the hemophilia A patients there is a formation of factor VIII inhibitors (i.e., antibodies against factor VIII), which inhibits the effect of the administered factor VIII preparations.

The treatment of factor VIII inhibitor patients is very difficult and associated with risks and previously there was only a limited number of special methods for treating

025791

these patients.

However, it is possible in the case of patients with a low FVIII inhibitor content to administer very expensive, high doses of factor VIII and to neutralize in this manner the factor VIII antibodies. The excess factor VIII then acts hemostatically. In many cases a desensitizing takes place and it is again possible to use standard factor VIII treatments. However, such a treatment with a high dosage requires large amounts of factor VIII, is time-consuming and can be associated with severe anaphylactic side reactions. Alternatively, the treatment can take place with porcine factor VIII molecules.

Another cost-intensive method for the removal of the factor VIII inhibitors works with the extra-corporal immunoadsorption on lectins which bond to immunoglobulins (protein A, protein G) or to immobilized factor VIII. Since the patient must be connected during this treatment to the apheresis machine, this method is also very stressful for the patient and it is not possible to treat an acute bleeding with it.

However, the therapy of choice was previously the administration of activated prothrombin complex concentrates (activated prothrombin complex concentrates, APCC) such as FEIBA® and AUTOPLEX® which is suitable even for patients with a high inhibitor titer for the treatment of acute bleedings (DE 31 27 318).

In the intravascular system of blood clotting the last step is the activation of factor X by factor IXa, a reaction which is stimulated by the bonding of the factor VIIIa to factor IXa. At this time a “tenase” complex is formed consisting of the factors IXa, VIIIa, X and phospholipid. Without the bonding of FVIIIa, fIXa shows no or only a very slight enzymatic activity against FX.

025791

A number of possible bonding sites of factor VIIIa to factor IXa was characterized in recent years and showed that antibodies or peptides which bond to these regions inhibits the activity of the FIXa (Fay et al., J. Biol. Chem., 1994, vol. 269, pp. 20522-20527, Lenting et al., J. Biol. Chem., 1996, vol. 271, pp. 1935-1940, Jorquera et al.,  
5 Circulation, 1992, vol. 86, abstract 2725).

An inhibition of clotting factors, among others also factor IX, was also be achieved by monoclonal antibodies with the goal of preventing the formation of thrombosis (WO 97/ 26010).

10

An opposite effect, namely the reinforcing of the activation of factor X brought about by factor IXa is described by Liles, D.K. et al., Blood, 1997, vol. 90, Suppl. 1, 2054) by the bonding of a factor VIII peptide (Amino acids 698-712). However, this effect only occurs in the absence of factor VIIIa, in the presence of factor VIIIa the  
15 splitting of factor X brought about by the factor IXa/factor VIIIa is inhibited by this peptide.

20

Particularly in view of the possible risks and side effects that can occur in the treatment of hemophilia patients, there is a great need for a possible therapy which  
also makes possible in particular an effective treatment of FVIII inhibitor patients. Therefore, a problem of this invention is to find a preparation for the treatment of blood clotting disturbances which also has special advantages for factor VIII patients.

25

The invention solves this problem in that antibodies or antibody derivatives against factor IX/factor IXa were produced which comprise a factor VIIIa cofactor activity and/or a an activity activating factor IXa and which result in an increasing of the procoagulant activity of the factor IXa.

025791

Surprisingly, the action of these antibodies and antibody derivatives which activate factor IX/factor IXa in accordance with the invention is not adversely affected by the presence of inhibitors such as, for example, inhibitors against factor VIII/factor VIIIa but even reinforces the procoagulant activity of the factor IXa in this case.

5

Another advantage of this invention is that the administration of the preparation according to the invention makes a rapid blood clotting possible even given a lack of the factor VIII or of factor VIIIa even if an FVIII inhibitor patient is concerned. These agents are also surprisingly effective in the presence of factor VIIIa.

10

The FVIII-like cofactor activity of the agents according to the invention is that the manner of acting of the agent is comparable to the manner of acting of factor VIIIa as regards the generation of factor Xa.

15 The antibodies or antibody derivatives according to the invention accordingly have an FVIII cofactor activity which have a ratio of background (background noise) to measured value of at least 3 in an FVIII assay (e.g. COATEST® test or immuno-chrome test) after two hours incubation. The calculation of this ratio can take place, e.g., according to the following scheme:

20

$$\frac{\text{antibody measured value (OD 405)} - \text{empty reagent value}}{\text{mouse- IgG measured value (OD 405)} - \text{empty reagent value}} \geq 3$$

25 after two hours incubation.

The antibodies according to the invention have an *in vivo* half-life of at least 5 days, preferably at least 10 days, especially preferably at least 20 days.

025791

Another aspect of this invention is a preparation containing antibodies and or antibody derivatives against factor IX/factor IXa and a pharmaceutically acceptable carrier substance. Furthermore, the preparation according to the invention can additionally contain factor IX and/or factor IXa.

5

The present invention also comprises the nucleic acids coding for the antibodies and antibody derivatives according to the invention, expression vectors, the hybridoma cell lines and methods of their preparation.

10 Antibodies are immunoglobulin molecules with a specific amino acid sequence which only bind to antigens which induce the synthesis (or its immunogen) and/or to antigens (or immunogen) which are very similar to the first ones. Each immunoglobulin molecule consists of two types of polypeptide chains. Each molecule consists of large, identical heavy chains (H chain) and two light, also  
15 identical chains (L chain). The polypeptides are connected via disulfide bridges and non-covalent bonds. In vivo, the heavy and light chains are formed on different ribosomes, joined together in the cell and secreted as intact immunoglobulins (Roitt, I. et al., in: Immunology, second ed., 1989).

20 The antibodies, antibody derivatives and organic compounds derived from them according to the invention comprise human and animal monoclonal antibodies or fragments of them, individual chain antibodies and mini-antibodies (fragments of individual chain antibodies), bispecific antibodies, "diabodies", triabodies" or di-, oligo- or multimers of them. Peptidomimetica or peptides which are derived from  
25 the antibodies according to the invention are also comprised, for example, they comprise one or more of the CDR regions, preferably the CDR3 region.

Completely human monoclonal antibodies are also comprised. Furthermore, peptide sequences which, based on a structure activity compound, were subjected to an

025791

artificial modelling process are also comprised (Greer, J., et al., J. Med. Chem., 1994, vol. 37, pp. 1035-1054.

The concept antibodies and antibody derivatives activating factor IXa can also  
 5 comprise proteins which are produced by expression of a changed, immunoglobulin-  
 coding region in a host cell, for example “technologically modified antibodies”, e.g.,  
 synthetic antibodies, chimera or humanized antibodies or their mixtures, or  
 antibody fragments which partially or entirely lack the constant region, e.g., Fv, Fab,  
 Fab’ or F (ab) ‘<sub>2</sub>, etc. For example, a part or parts of the light and/or heavy chain  
 10 can be replaced in these technologically modified antibodies. For example, such  
 molecules can comprise antibodies which consist of a humanized, heavy chain and  
 of an unmodified, light chain (or chimera light chain or vice versa. The concepts  
 Fv, Fc, Fd, Fab, Fab’ or F (ab) <sub>2</sub> are used as described in the prior art. (Harlow, E.  
 and Lane, D. in “Antibodies, a Laboratory Manual), Cold Spring Harbor Laboratory,  
 15 1988).

The present invention also comprises the use of Fab fragments or F (ab) <sub>2</sub> fragments  
 stemming from monoclonal antibodies (mAb) which are directed against factor  
 IX/factor IXa and bring about an increase of the procoagulant activity of the factor  
 20 IXa.

The heterological framework regions and constant regions are preferably selected  
 from the human immunoglobulin classes and isotypes such as IgG (subtypes 1 to 4),  
 IgM, IgA and IgE. During the course of the immuno reaction a “class switch” of the  
 25 immunoglobulins can also take place, for example, a switch from IgM to IgG, during  
 which the constant regions are exchanged, e.g. from  $\mu$  to  $\gamma$ . A class which can also  
 be induced in a quite purposeful manner by technological gene methods (directed  
 class switch recombination”) such as is known from the prior art (Esser, C. and  
 Radbruch, A., Annu. Rev. Immunol., 1990, vol. 8, pp. 717-735).



025791

However, the antibodies and antibody derivatives according to the present invention must not comprise only human sequences of the immunoglobulin proteins.

5 In a particular embodiment a humanized antibody contains “complement determining regions” (CDRs) consisting of murine monoclonal antibodies which are inserted into the framework areas of selected human antibody sequences. However, even human CDR regions can be used. The variable regions in the human light and heavy chains are preferably technically changed by one or more CDR exchanges. It  
10 is also possible to use all six CDRs or very different combinations of less than six CDRs.

The humanized antibody according to the present embodiment preferably has the structure of a human antibody or of a fragment of it and comprises the combination  
15 of qualities which are necessary for an efficient therapeutic usage, i.e., e.g., the treatment of clotting disturbances in patients, preferably of factor VIII inhibitor patients.

A chimera antibody differs from the humanized antibody in that it contains the  
20 entire variable regions including the framework regions of the heavy and light chains of non-human origin in combination with the constant regions of both chains from human immunoglobulin. For example, a chimera antibody consisting of murine and human sequences can be produced.

25 According to the present invention the antibodies and antibody derivatives can also be individual chain antibodies (“single-chain antibody”) or mini-antibodies (scFv fragments which are linked, for example, with proline-rich sequences and oligomerizing domains, see Pluckthun, A. and Pack, P., Immunotechnology, 1997, vol. 3, pp. 83-105) or individual chains Fv (sFv) which incorporate the entire

025791

antibody bonding region in a single polypeptide chain. For example, the individual chain antibodies can be represented by linking the V genes with an oligonucleotide which was constructed as a linker sequence and connects the C terminal of the first V region to the N terminal of the second V region, for example in the arrangement

5  $V_H$ -linker- $V_L$  or  $V_L$ -linker- $V_H$ ; therefore,  $V_H$  as well as  $V_L$  can represent the N-terminal domains. (Huston JS et al., Int. Rev. Immunol., 1993, vol. 10, pp. 195-217; Raag, R. and Whitlow, M., FASEB, J., 1995, vol. 9, pp. 73-80). The protein that can be used as a linker sequence can have, for example, a length of up to 150 Å, preferably up to 80 Å, especially preferably up to 40 Å. Linker sequences which

10 contain glycine and serine or glutamine and lysine on account of the solubility are especially preferred on account of their flexibility. The selection of the amino acids also takes place according to the criteria of immunogenicity and stability even independently of whether these individual chain antibodies should be suitable for physiological or industrial applications (e.g., immunoaffinity chromatography). The

15 individual chain antibodies can also be present as aggregates, for example as trimers, oligomers or multimers.

However, the linker sequence can also be lacking and the linking of the  $V_H$  and  $V_L$  chains can take place directly.

20

Bispecific anti-bodies are macromolecular, heterobifunctional “cross linkers” with two different bonding specificities inside an individual molecule. This group includes, for example, bispecific (bs) IgGs, bs IgM-IgAs, bs IgA-Dimers, bs (Fab')<sub>2</sub>, bs(scFv)<sub>2</sub>, Diabodies, bs bis Fab Fc etc. (Cao Y. and Suresh M.R., Bioconjugate Chem., 1998, vol. 9, pp. 635-644).

25

The term “peptidomimetica: denotes protein components with a low molecular weight which imitates the structure of a natural peptide component or of confirmation templates “(templates”) which induce a specific structural formation

025791

in an adjacent peptide sequence (Kemp, DS, Trends Biotechnol. 1990, pp. 249-255).  
The peptidomimetica can stem, for example from CDR3 domains.

The concept antibodies and antibody derivatives can also comprise agents which  
5 were obtained by a determination of data on the structure-activity relationship. These  
compounds can also be used as peptidomimetics (Grassy, G. et al., Nature  
Biotechnol., 1998, vol. 16, pp 748-752; Greer, J. et al., J. Med. Chem., 1994, vol.  
37, pp. 1035-1054).

10 Examples for the antibodies or antibody derivatives according to the invention were  
deposited on 9 September, 1999 under the numbers 99090924 (#198/A1), 99090925  
(#198/B1) and 99090926 (#198/BB1) according to the Budapest Treaty.

#### **METHOD FOR THE PRODUCTION:**

15

The antibodies of the present invention can be produced by the methods known from  
the prior art, for example by conventional hybridoma techniques or by phage display  
gene banks, immunoglobulin chain “shuffling” or humanizing techniques (Aharlow,  
B. and Lane D. in: Antibodies, a Laboratory Manual, Cold Spring Harbor  
20 Laboratory, 1988).

The production of the antibodies and antibody derivatives according to the invention  
can take place by any method known from the prior art. For example, by  
conventional hybridoma techniques (Antibodies, a Laboratory Manual, Cold Spring  
25 Harbor Laboratory, 1988, eds. Harlow and Lane, pp. 148-242). According to the  
present invention human or also non-human species can be used for this such as, for  
example, cattle, pigs, monkeys, chickens or rodents (mice, rats). For example,  
normally immunocompetent Balb/c mice or FIX-deficient mice can be used. (The  
factor IX-deficient mice stem from Dr. Darrel Stafford of the University of North

025791

Carolina, Chapel Hill). The immunization can take place here, for example, by factor IX, factor IX $\alpha\alpha$  or completely activated factor IX $\alpha\beta$  or with fragments of them.

The hybridomas are selected in such a manner that the antibodies and antibody derivatives -in the supernatants of the hybridoma cells bond to factor IX/factor IX $\alpha$  and bring about an increase of the procoagulant activity of the factor IX $\alpha$ . The increase of the procoagulant activity can be demonstrated, for example, by test methods such as those known from the prior art for the measuring of factor VIII-like activity, for example, chromogenic assays.

Alternatively to the above, the antibodies and antibody derivatives according to the invention can also be produced by recombinant production methods. The DNA sequence of the antibodies according to the invention can be determined in them by known techniques and parts or the totality of the antibody DNA can be expressed in suitable systems. Recombinant production methods can be used such as, for example, the using of phage display, synthetic and natural libraries; expression of the antibody proteins in known expression systems, expression in transgenic animals, etc. (Jones et al., Nature, 1986, vol. 321, pp. 522-525; Phase Display of Peptides and Proteins, A Laboratory Manual, 1996, eds. Kay et al., pp. 127-139; US 4, 873, 316).

The expression of the recombinantly produced antibodies can take place by conventional expression vectors such as, for example, bacterial vectors such as pBR322 and its derivatives, pSKF or eukaryotic factors such as pMSG, SV40 vectors, etc. Those sequences coding for the antibody can be provided with regulatory sequences which regulate the replication and expression and furthermore the secretion from the host cell. These regulatory sequences comprise promoters, e.g., CMV or SV40; and signal sequences.

025791

The expression vectors can also contain selection markers and amplification markers such as the dihydrofolate reductase gene (DHFR), hygromycin B-phosphotransferase, thymidine kinase, etc.

- 5 The components of the vectors used such as selection markers, replicons, enhancers, etc. can either be commercially acquired or produced by conventional methods. The vectors can be constructed for the expression in very different cell cultures, e.g., for mammalian cells such as CHO, COS; fibroblasts, insect cells, yeast or bacteria such as E. coli, etc. Those cells are preferably used which allow an optimal glycosylation
- 10 of the expressed protein. The vector pBax (see fig. 17), which is expressed in CHO cells or SK-Hep, is especially preferred.

The production of Fab fragments or F (ab)<sub>2</sub> fragments can take place according to method like those known from the prior art, e.g., by splitting of mAb with proteolytic

15 enzymes such as papain and/or pepsin, or by recombinant methods.

These Fab fragments and F (ab)<sub>2</sub> fragments can also be produced by a phage display gene bank (Winter et al., 1994, Ann. Rev. Immunol., 12:433-455).

- 20 The antibody derivatives can also be produced by methods like those known from the prior art, for example by molecular modelling, e.g., from Grassy, G. et al., Nature Biotechnol., 1998, vol. 16, pp. 748-752, or Greer, J. et al., J.Med.Chem., vol. 37, pp. 1035-1054, or Rees, A. et al., in: "Protein Structure Prediction. A practical approach", ed. Sternberg, M.J.E., IRL press, 1996, chapt. 7-10, pp. 141-261.

25

The purification of the antibodies and antibody derivatives according to the invention can also be carried out by methods like those described in the prior art, e.g., ammonium sulfate precipitation, affinity purification (protein G-sepharose), ion exchange chromatography, gel chromatography, etc.

025791

For example, the following methods can be used as test methods in which it is demonstrated that the produced antibodies and antibody derivatives bond to factor IX/factor IXa increase the procoagulant activity of the factor IXa and have an activity similar to factor VIII: One stage clotting test (Mikaelsson and Oswaldson, Scand. J. Haematol. Suppl., 33,k pp. 79-86, 1984) or chromogenic tests such as COATEST VIII:C® (Chromogenix) or immunochrom (IMMUNO). In principle, all methods can be used like those also use for determining the factor VIII activity. Non-specific mouse-IgG-antibodies can be used as blank reading check for the measurements.

The present antibodies and antibody derivatives are suitable for being therapeutically used in the treatment of clotting disturbances, e.g., in hemophilia A, factor VIII inhibitor patients, etc. The administration can take place by any method which is suitable for rendering the therapeutic agent effective for being used in patients, e.g., by administration such as, for example, orally, subcutaneously, intramuscularly, intravenously or intranasally.

Therapeutic means according to the invention can be produced as preparations containing a sufficient amount of antibodies or antibody derivatives as active agent in a pharmaceutically acceptable carrier substance. These means can be present in liquid as well as in powdery form. However, furthermore, the preparations according to the invention can also contain mixtures of different antibodies, their derivatives and/or organic compounds derived from them or also mixtures consisting of antibodies and factor IX and/or factor IXa. Factor IXa can be present here as factor IXa $\alpha$  and/or factor IXa $\beta$ . Aqueous carrier substance can be, for example, saltern. The solutions are sterile and the sterilization takes place by conventional methods.

The antibodies or antibody derivatives according to the invention can be present

025791

lyophilized for storage and be suspended in a suitable solvent before administration. This method proved to be generally advantageous for the conventional immunoglobulins and known lyophilizing methods and reconstitution methods can be used in it.

5

Moreover, the antibodies and antibody derivatives can also be used for industrial applications, for example for the purification of factor IX/factor IXa by affinity chromatography, or as a component of demonstration methods (e.g., ELISA assays), or as agent for the identification of and interaction with functional domains from a target protein.

10

The present invention is described in detail by the following examples and drawing figure but is of course not limited by them:

15 The figures show:

Fig. 1. Results of a screening on FVIII-like activity of supernatants from hybridoma cell cultures. Preselected clones from fusion experiments, #193, #195 and #196 were tested in a chromogenic assay.

20

Fig. 2: Result of a screening of an IgG-mediated factor VIII-like activity in supernatants of a hybridoma cell culture of a “master” plate.

Fig. 3: Subcloning of the clone 193/CO, results of the first cloning round.

25

Fig. 4: Comparison of the chromatographic FVIII -like activity and factor IX-ELISA reactivity of the hybridoma cultures, derived from the initial clone 193/CO.

Fig. 5: Results of the measuring of the chromatographic activity of a few master

025791

clones and subclones.

Fig. 6A: FVIII-like activity of anti-FIX/FIXa antibodies 193/AD3 and 196/AF2.

- 5 Fig. 6B: Comparison of the chromatographic activity of factor FVIII, 196/AF1, 198/AC1/1 and mouse IgG.

Fig. 7A: Comparison of the kinetics of the factor Xa generation by factor FVIII and 196/AF2 with and without the addition of a factor Xa specific inhibitor.

10

Fig. 7B: Comparison of the kinetics of the factor Xa generation by factor FVIII, mouse IgG and anti-factor IX/IXa-antibodies 198/AM1 with and without the addition of a factor Xa specific inhibitor.

- 15 Fig. 8A: Measuring the dependence of the factor FVIII-like activity of purified antifactor IX/IXa-antibody 198/AC1/1 in the presence in the absence of phospholipids, FIXa/FX and calcium ions.

- Fig. 8B: Measuring the dependence of the FXa generation by anti-FIXa antibody  
20 196/AF1 on the presence of phospholipids,  $\text{Ca}^{2+}$  in FIXa/FX.

Fig. 8C: Measuring the FXa generation by non-specific mouse IgG antibodies.

- Fig. 9: Graphic representation of the clotting times of factor FVIII-deficient plasma  
25 in an APTT assay using different concentrations of anti-factor IX/IXa antibody 193/AD3.

Fig. 10A: Antibody 193/AD3 results given the presence of factor IXa in a reduction of the clotting time of factor FVIII-deficient plasma.



025791

Fig. 10B: Dosage-dependent reduction of the clotting time by the anti-body 193/AD3 in the presence of factor IXa- and factor FVIII inhibitors.

5 Fig. 11: Chromogenic activity of the antibodies 198/A1, 198/B1 and 198/AP1 in the presence and the absence of human FIXa.

Fig. 12: Primer sequences for the amplification of the genes of the variable, heavy chain of the mouse antibody.

10

Fig. 13: Primer sequences for the amplification of the genes of the variable, light (kappa-) chain of the mouse antibody.

Fig. 14: DNA and derived protein sequence of the scFv from the hybridoma cell line 193/AD3.

15

Fig. 15: DNA and derived protein sequence of the scFv from the hybridoma cell line 193/K2.

Fig. 16: DNA and derived protein sequence of the scFv from the hybridoma cell line 198/B1/1.

20

Fig. 17: Vector pBax-IgG1.

**Examples :**

---

**Example 1: Immunization of immunocompetent mice and generation of anti-FIX/IXa antibody secreting hybridoma cells**

Groups of 1-3 normal immunocompetent 5-8 weeks old Balb/c mice were immunized with 100µg antigen (100µl doses) via the i.p. route. In a typical experiment mice were inoculated with either recombinant human coagulation factor (F) IX (Benefix™), human activated (FIXa) FIXα (Enzyme Research Laboratories, Lot:

05791

HFIXa $\alpha$  1190L) or human FIXa $\beta$  (Enzyme Research Laboratories, Lot: HFIXAa $\beta$  1332 AL,) adjuvanted with Al(OH)<sub>3</sub> or KFA.

Individual mice were boosted at various times with 100 $\mu$ g antigen (100 $\mu$ l doses, i.p) and sacrificed two days later. Spleen cells were removed and fused to P3 X63-Ag8 6.5.3 myeloma cells essentially as described by Lane et al., 1985 (J. Immunol. Methods, vol 81, pp. 223-228). Each fusion experiment was individually numbered i.e. #193, 195, 196 or 198.

Hybridoma cells were grown in 96 well plates on a macrophage feeder layer (app. 10<sup>5</sup> cells/ml) and selected in HAT-medium (RPMI-1640 medium supplemented with antibiotics, 10% FCS, Na-pyruvate, L-glutamine, 2-mercaptoethanol and HAT (HAT 100x: 1,0x10<sup>-2</sup> M hypoxanthine in H<sub>2</sub>O (136,1 mg/100ml H<sub>2</sub>O), 4,0x10<sup>-5</sup> M aminopterin in H<sub>2</sub>O (1,76 mg/100ml H<sub>2</sub>O) and 1,6x10<sup>-3</sup> M thymidine in H<sub>2</sub>O (38,7 mg/100ml H<sub>2</sub>O). Medium was first changed after 6 days and thereafter twice a week. After 2-3 weeks HAT-medium was changed to HT-medium (RPMI-1640 supplemented with antibiotics, 10%FCS, Na-pyruvate, L-glutamine, 2-mercaptoethanol and HT) and later on (after additional 1-2 weeks) to normal growth medium (RPMI-1640 medium supplemented with 10%FCS, Na-pyruvate, L-glutamine and 2-mercaptoethanol) (see: HYBRIDOMA TECHNIQUES, EMBO, SKMB Course 1980, Basel).

In another set of experiments FIX deficient C57Bl6 mice (Lin et al., 1997, Blood, 90:3962) were used for immunization and subsequent hybridoma production. Since FIX k.o. mice do not express endogenous FIX, the anti (a)-FIX antibody spectrum achievable is supposed to be different compared to normal Balb/c mice (lack of tolerance).

---

**Example 2: Assay of FVIII-like activity in supernatants of anti-FIX/FIXa antibody secreting hybridoma cells**

In order to assay the FVIII-like activity of anti-FIXa antibodies secreted by hybridoma cells, the commercially available test-kit COATEST VIII:C/4® (Chromogenix) was employed. The assay was done essentially as described by the manufacturer with the following modifications:

025791

To allow a high throughput screening, the assay was downscaled to microtiter plate format. Briefly, 25  $\mu$ l aliquots of hybridoma supernatants were transferred to microtiter plate (Costar, #3598) wells and warmed to 37°C. Chromogenic substrate (S-2222), synthetic thrombin inhibitor (I-2581), factor (F) IXa and FX were reconstituted in sterile water and FIXa/FX mixed with phospholipids according to the suppliers protocol. Per reaction, 50 $\mu$ l of the phospholipid/FIXa/FX solution were combined with 25 $\mu$ l  $\text{CaCl}_2$  (25mM) and 50 $\mu$ l of the substrate/inhibitor cocktail. To start the reaction, 125 $\mu$ l of the premix were added to the hybridoma supernatant in the microtiter plates and incubated at 37°C. Absorbency at 405nm and 490nm of the samples was read at various times (30min to 12h) against a reagent blank (MLW, cell culture medium instead of hybridoma supernatant) in a Labsystems iEMS Reader MF™ microtiter plate reader. FVIII-like activity of the samples was calculated by comparing the absorbency of the samples against the absorbency of a diluted FVIII reference standard (IMMUNO AG # 5T4AR00) using GENESIS™ software.

The results of a screening for FVIII-like activity in hybridoma cell culture supernatants are shown in Fig. 1. Pre-selected clones derived from fusion experiments #193, #195 and #196 (see above) were examined in a chromogenic FVIII assay as described. Clones 193/M1, 193/N1 and 193/P1 are subclones derived from the master clone 193/C0 (see below). Master clone 195/I0 was derived from fusion experiment #195 and clones 196/A0, 196/B0 and 196/C0 were derived from fusion experiment #196. In a typical screening experiment, approximately 1000 clones (in 96 wells) from a single fusion experiment were pre-screened for FVIII-like activity. Subsequently, selected clones were grown on a larger scale (3-5ml supernatant) and re-analyzed in a chromogenic assay. As a negative control cell culture medium was assayed on each plate (MLW).

Wells either exhibiting high FVIII-like activity in the first place or substantial FVIII-like activity on a second occasion were subjected to subcloning procedures. The selection and subcloning process is exemplified for the screening and subcloning of an IgG producing cell line (i.e. 193/C0) but has

025791

been done exactly the same way for an IgM (i.e. 196/CO, see below, Fig. 5) producing clone.

The selection process was done by initially plating all hybridoma cell clones derived from a single fusion experiment on ten 96 well plates thereby creating the so called "master plates". Singular positions (wells) on a master plate usually do contain more than one hybridoma cell clone (usually 3 to 15 different clones). Subsequently, the antibody secreted by only several 1000 cells is tested. These cells grow under conditions suboptimal for antibody production, which is known to be best in dying cells. So the expected specific anti-FIX antibody concentration in the supernatant may be in the range of  $10^{-12}$  to  $10^{-14}$  M. This also explains why incubation periods have to be extended compared to standard FVIII assays.

Results of a screening for a IgG mediated FVIII-like activity in hybridoma cell culture supernatants of a master plate are shown in Fig. 2. Supernatants were examined in a chromogenic FVIII assay. Shown are the results derived from the fifth master plate of fusion experiment number #193 (Balb/c mice immunized with FIX $\alpha$ ). Absorbency was read after 4 hours of incubation at 37°C. Position E5 was identified to exhibit FVIII like activity significantly higher than the blank (MLW). This cell pool was designated 193/C0 and was further subcloned (Figure 3). As each well of the master plate contains more than one hybridoma cell clone, cells of a single positive well were expanded and plated at a calculated cell density of 2 - 0.2 cells/well on a 96 well plate. Again, the supernatants were tested for FVIII-like activity and positive positions subjected to another round of subcloning. Typically three to four rounds of subcloning were performed with each clone displaying FVIII-like activity to obtain homogenous cell populations. Here the results of the chromogenic assay of the 193/C0 subclones are shown. Absorbency was read after an 4 hours incubation period at 37°C. Positions A6 and D5 exhibited substantial FVIII-like activity and were named 193/M1 and 193/P1, respectively. These two clones were subjected to another round of subcloning. As a negative control plain cell culture medium was assayed on each plate (MLW(H1)).

05791

A comparison of chromogenic FVIII-like activity and FIX-ELISA reactivity of small scale (3ml) hybridoma cultures is shown in Fig. 4. Before a decision was made whether a master clone (or subclone) was further subcloned, clones were grown in a 3-5 ml scale and the supernatants were checked again. This graph shows the FIX specific ELISA results and the FVIII-like chromogenic activity of the master clone 193/C0 and all its subclones which were identified as positives and re-checked. Blanks (absorbency of the chromogenic reagent itself) were subtracted in the case of the ELISA as well as the chromogenic assay readings depicted here. Clone 193/M1 was subcloned and yielded clones 193/V2, 193/M2 and 193/U2. The other clones of the 2<sup>nd</sup> round came from 193/P1, 193/AB2 and 193/P2 were subcloned. 193/AF3, 193/AB3 and 193/AE3 are subclones of 193/AB2, the other clones of the 3<sup>rd</sup> round come from 193/P2. Finally 193/AF3 (→193/AF4), AE3 (→193/AE4, 193/AL4, 193/AN4 and 193/AO4) and 193/AD3 (→193/AG4, 193/AH4, 193/AD4, 193/AI4, 193/AK4) were subcloned.

From each fusion experiment several (5-15) master clones (selected from the master plate) were identified and subjected to subcloning. Mostly after 3 rounds of subcloning the cell lines were homogenous as demonstrated by ELISA and chromogenic activity analysis (see Fig. 4) as well as by cDNA sequence analysis. A specific master clone and all its subclones produce the same FIX/FIXa binding antibody however, there are huge differences in the antibody protein sequences of clones derived from different master clones (see Example 11). Most hybridoma cell lines express antibodies from the IgG subclass (i.e. clones #193, #198, like 198/A1, 198/B1, 198/BB1). However, we were also able to select some clones expressing IgM antibodies.

---

The chromogenic activity of hybridoma supernatant of some important master clones and subclones. Absorbancies were measured after an 1h 30 min and 3h 30 min incubation period at 37°C (Fig. 5). In contrast to all the clones from the 193<sup>st</sup> fusion, clone 196/C0 and its subclone 196/AP2 produce a FIX/FIXa specific IgM antibody that gives a strong chromogenic activity even after a short period of incubation.

05791

To do a more in depth analysis of the biochemical properties of certain antibodies, homogenous hybridoma cell lines expressing different antibodies with FVIII-like activity were expanded and used to express the antibody in question on a larger scale (100-1000ml). These antibodies were affinity purified (see Example 3) prior to further experiments.

**Example 3: Factor IX/FIXa<sub>(α,β)</sub> binding properties of antibodies exhibiting FVIII-like activity**

Factor IX and the two activated forms of FIX, FIXα and FIXαβ (FIX/FIXa<sub>(α,β)</sub>) were diluted in TBS (25mM Tris HCl, 150mM NaCl, pH 7.5) to a final concentration of 2μg/ml. Nunc Maxisorp ELISA plates were coated with 100μl FIX/FIXa<sub>(α,β)</sub> solution according to standard procedures (4°C, overnight) and washed several times with TBST (TBS, 0.1% (v/v) Tween 20). 50μl hybridoma supernatant was diluted 1:1 with 50μl TBST/2%BSA and added to the coated ELISA plate. After an incubation period of 2h at room temperature (RT), plates were washed 4 times with TBST and incubated (2h, RT) with 100μl /well of a 1:25000 dilution (in TBST/1%BSA) of an anti-mouse IgG (Fc-specific) peroxidase conjugated antibody (Sigma, #A-0168). Wells were washed 5 times with TBST and finally stained with 100μl freshly prepared staining solution (10ml 50mM sodium citrate, pH 5 supplemented with 100μl OPD (60mg OPD/ml) and 10μl 30% H<sub>2</sub>O<sub>2</sub>). The reaction was stopped by the addition of 50μl H<sub>2</sub>SO<sub>4</sub> and the optical density recorded at 492nm and 620nm in a Labsystems iEMS Reader MF™ microtiter plate reader employing GENESIS™ software.

In certain cases instead of an anti-mouse IgG ELISA, an anti-mouse IgM ELISA was carried out.

---

**Purification of mouse-IgG from hybridoma cell culture supernatants:**

Hybridoma supernatant (100-500 ml) was supplemented with 200mM Tris/HCl buffer (pH 7.0) and solid NaCl to give final concentrations of 20mM Tris and 3M NaCl, respectively. The supernatant was then clarified by centrifugation at 5500xg for 10 minutes. A 1ml protein G affinity chromatography column

05791

(Protein G Sepharose Fast Flow, Amersham-Pharmacia) was washed with 15 ml 20mM Tris/Cl pH 7.0 and afterwards equilibrated with 10 ml of 20mM Tris/Cl buffer pH 7.0 containing 3M NaCl. The hybridoma supernatant containing 3M NaCl was then loaded onto the column by gravity. Column was washed with 15 ml of 20mM Tris/Cl buffer pH 7.0 containing 3M NaCl. Bound IgG were further eluted with 12ml glycine/HCl buffer pH 2.8 and 1ml fractions were collected. 100 $\mu$ l of 1M Tris pH 9.0 were added to each fraction for neutralization. Fractions containing the IgG were identified by mixing 50 $\mu$ l with 150 $\mu$ l of a staining solution (BioRad concentrate, 1:5 diluted with water) in wells of a microplate. Positive fractions were pooled, concentrated to 1ml in a ultrafiltration concentrator device (Centricon Plus 20, Amicon) according to the manufacturer. The concentrate was diluted with 19 ml TBS (20mM Tris/Cl buffer pH 7.0 containing 150mM NaCl) and again concentrated to 1ml. The diluting-concentrating step was repeated for two more times in order to bring IgG into TBS.

#### **Purification of mouse-IgM from hybridoma cell supernatants:**

100-500 ml of hybridoma cell culture supernatant were concentrated to 5-10ml either with a ultrafiltration concentrator device (Centricon Plus 20, Amicon) according to the manufacturer or by ammonium sulfate precipitation (40% saturation, 0°C) and redissolving the precipitate with 5-10ml of TBS. In either case the concentrate was dialyzed against 20mM Tris Cl buffer pH 7.4 containing 1.25M NaCl and further concentrated to 1ml in a Centricon Plus 20, (Amicon) ultrafiltration device. IgM were purified from this concentrate with the ImmunoPure IgM Purification Kit (Pierce) according to the manufacturer. Fractions collected during elution from the maltose binding protein-column were tested for IgM, pooled, concentrated and brought into TBS as described for IgG.

#### **Determination of IgG concentrations in purified preparations:**

##### **Total IgG content:**

025791

280nm - extinction of appropriate dilutions were measured. E280 = 1.4 corresponds to 1mg/ml protein.

**Factor IXa specific IgG (quantitative ELISA):**

Wells of a microplate (Nunc Maxisorp) were incubated with 2µg/ml factor IXa diluted in TBS (25mM Tris/HCl pH 7.5 containing 150mM NaCl) over night at 4°C. Wells were washed four times with TBST (25mM Tris/HCl pH 7.5 containing 150mM NaCl and 0.1% (v/v) Tween 20). As a standard monoclonal AB the HIX1 anti FIX (accurate) is used. Standard and samples were diluted in TBST containing 2%(w/v) BSA. The standard dilution series and appropriate dilutions of the samples were incubated on the ELISA-plate for 2 hours at room-temperature. Plates were washed 4 times with TBST and incubated (2h, RT) with 100µl /well of a 1:25000 dilution (in TBST/1%BSA) of an anti-mouse IgG (Fc-specific) peroxidase conjugated antibody (Sigma, #A-0168) FIXa. Wells were washed 5 times with TBST and finally stained with 100µl freshly prepared staining solution (10ml 50mM sodium citrate, pH 5 supplemented with 100µl OPD (60mg OPD/ml) and 10µl 30% H<sub>2</sub>O<sub>2</sub>). The reaction was stopped by the addition of 50µl H<sub>2</sub>SO<sub>4</sub> and after 30 minutes the optical density recorded at 492nm and 620nm in a Labsystems iEMS Reader MF™ microtiter plate reader employing GENESIS™ software.

**Example 4: Anti-FIX/FIXa antibodies exhibiting FVIII-like activity in a chromogenic FVIII assay**

Several anti-FIX/FIXa antibody producing hybridoma clones were subcloned up to four times and the resulting monoclonal hybridoma cell line used to produce monoclonal antibody containing supernatant. IgG isotype antibodies derived from these supernatants were purified over affinity columns and dialyzed against TBS (see above). IgM antibodies were just used as unpurified supernatant fractions. The following experiments were done with two sets of representative antibodies: 193/AD3 and 198/AC1/1 (IgG isotyp) and 196/AF2 and 196/AF1 (IgM isotyp) (Fig. 6A and Fig. 6B). Briefly, 25µl aliquots of monoclonal antibody containing sample (unpurified hybridoma supernatant or, where indicated, a certain amount of FIX specific antibody) were



025791

transferred to microtiter plate wells and warmed to 37°C. Chromogenic substrate (S-2222), synthetic thrombin inhibitor (I-2581), factor (F) IXa and FX were reconstituted in sterile water, and FIXa/FX mixed with phospholipids according to the suppliers protocol. Per reaction, 50µl of the phospholipid/FIXa/FX solution were combined with 25µl CaCl<sub>2</sub> (25mM) and 50µl of the substrate/inhibitor cocktail. To start the reaction, 125µl of the premix were added to the monoclonal antibody solution in the microtiter plates and incubated at 37°C. Absorbency at 405nm and 490nm of the samples was read at various times (5min to 6h) against a reagent blank (cell culture medium instead of hybridoma supernatant) in a Labsystems iEMS Reader MF™ microtiter plate reader using GENESIS™ software.

The time course of chromogenic FVIII-like activity exhibited by monoclonal antibodies 193/AD3 (IgG isotype) and 196/AF2 (IgM isotype) compared to human FVIII (12 and 16mU/ml), TBS and to cell culture medium is shown in Fig. 6A. After a lag phase, both antibodies give rise to chromogenic substrate cleavage, as judged by the increasing optical density measurable at 405nm wavelength.

The time course of chromogenic FVIII-like activity exhibited by monoclonal antibodies 198/AC1/1 (IgG isotype, 10µg/ml) and 196/AF1 (IgM isotyp, unpurified supernatant) compared to human FVIII (16mU/ml) and 10µg/ml of mouse IgG is shown in Fig. 6B. After a lag phase, both antibodies give rise to chromogenic substrate cleavage, as judged by the increasing optical density measurable at 405nm wavelength.

---

**Example 5: FVIII-like activity exhibited by anti FIX/FIXa-antibodies generates factor Xa and is phospholipid, FIXa/FX and Ca<sup>2+</sup> dependent.**

Factor VIII activity is usually characterized in a chromogenic and/or in a APTT based clotting assay. Both types of assays relay on the FVIIIa/FIXa mediated factor Xa generation. In the case of a chromogenic FVIII assay the factor Xa produced will subsequently react with a chromogenic substrate, which can be

025791

monitored spectroscopically i.e. in an ELISA reader. In an APTT based clotting assay free factor Xa will assemble with FVa on a PL surface in the so called prothrombinase complex and activate prothrombin to thrombin. Thrombin in turn gives rise to fibrin generation and finally to the clot formation. Central to the two assay systems is the factor Xa generation by the FVIIIa/FIXa complex.

To demonstrate that the FVIII-like activity exhibited by anti-FIX/FIXa-antibodies indeed generates factor Xa the following experiment was carried out:

Several 25 $\mu$ l aliquots of the (unpurified) hybridoma supernatant 196/AF2 (IgM isotype) were transferred to microtiter plate wells and warmed to 37°C. As a positive control, 16mU of Recombinate™ were diluted into hybridoma medium (196 HM 007/99) and treated exactly the same way as the hybridoma supernatant. As a negative control, plain hybridoma medium was used. Chromogenic substrate (S-2222), synthetic thrombin inhibitor (I-2581), factor (F) IXa and FX were reconstituted in sterile water and FIXa/FX mixed with phospholipids according to the suppliers protocol. Pefabloc Xa® a factor Xa specific proteinase inhibitor (Pentapharm, LTD) was reconstituted with water to a final concentration of 1mM/l. Per reaction, 50 $\mu$ l of the phospholipid/FIXa/FX solution were combined with 25 $\mu$ l CaCl<sub>2</sub> (25mM) and 50 $\mu$ l of the substrate/thrombin-inhibitor cocktail. To start the reaction, 125 $\mu$ l of the premix were added to the samples in the microtiter plates and incubated at 37°C. Where indicated 35 $\mu$ M Pefabloc Xa® were added. Absorbency at 405nm and 490nm was read at various times (every 5 minutes to 6h) against a reagent blank (cell culture medium) in a Labsystems iEMS Reader MF™ microtiter plate reader employing the GENESIS™ software.

The results of the factor IXa stimulation by the FVIII-like activity exhibited by the IgM anti FIX/FIXa-antibody 196/AF2 generates factor Xa as judged by the readily measurable cleavage of the chromogenic substrate S-2222 (compare "16mU FVIII" and "196/AF2") is shown in Fig. 7A. Factor Xa activity is effectively blocked by the FXa specific inhibitor "Pefabloc

025791

Xa®" (compare "196/AF2" versus "196/AF2 35 $\mu$ M Pefabloc Xa®") indicating that indeed FXa is generated.

The same experiment was performed using purified IgG-preparations of clone 198/AM1 (Fig. 7B). Purified IgG were diluted in TBS to a final concentration of 0,4mg/ml and 25 $\mu$ l (i.e. a total of 10 $\mu$ g) were transferred to microtiter plate wells and warmed to 37°C. As a positive control 6mU plasma-derived FVIII was used. 10 $\mu$ g unspecific mouse IgG (Sigma, I-5381) served as a negative control. Assay was performed as described above.

Further experiments show the factor IXa stimulation by the FVIII-like activity exhibited by the IgG anti FIX/FIXa-antibody 198/AM1 generates factor Xa as judged by the readily measurable cleavage of the chromogenic substrate S-2222 (Fig. 7B). Again factor VIII and antibody 198/AM1 generate FXa which is effectively blocked by the FXa specific inhibitor "Pefabloc Xa®". As a negative control unspecific mouse IgG (Sigma, I-5381) was assayed.

In another set of experiments the dependence of the FVIII-like activity on either purified anti FIX/FIXa-antibodies (IgM, Fig. 8A) or of unpurified antibodies derived from cell culture supernatants (IgG, Fig. 8B) on the presence of phospholipids (PL), FIXa/FX and Ca<sup>2+</sup> could be demonstrated. Mouse IgG was used as a control (Fig. 8C). Factor VIII-like activity was assayed essentially as described above. When indicated, either the FIXa/FX mixture, the PL or Ca<sup>2+</sup> was omitted from the reaction. Absorbency at 405nm and 490nm of the samples was read at various times against a reagent blank (buffer instead of purified antibody) in a Labsystems iEMS Reader MF™ microtiter plate reader. The results are shown in Fig. 8A, Fig. 8B and Fig. 8C.

The dependence of the FVIII-like activity of purified anti FIXa-antibody 198/AC1/1 (IgG isotype, concentration used throughout the assay was 10 $\mu$ g/ml) on the presence of phospholipids (PL), FIXa/FX and Ca<sup>2+</sup> is further shown in Fig. 8A. As easily recognizable, only the complete assay, including antibody, PL, Ca<sup>2+</sup>, FIXa/FX gives rise to a reasonable FXa generation.

05791

The dependence of the FVIII-like activity of cell culture supernatant containing unpurified IgM isotype anti FIX/FIXa-antibody (196/AF1) on the presence of phospholipids, FIXa/FX and  $\text{Ca}^{2+}$  is shown in Fig. 8B.

Again, as already shown for the purified IgG preparation (Fig. 8A), antibody 198/AC1/1, only the complete assay, including PL,  $\text{Ca}^{2+}$ , FIXa/FX, will give a reasonable amount of FXa generation. To demonstrate the specificity of the reaction, total IgG prepared from normal mouse plasma was assayed under the same conditions as above. The results are shown in Fig. 8C: No FVIII-like activity could be detected. There is, as expected no activity detectable in the absence of phospholipids, FIXa/FX and  $\text{Ca}^{2+}$ . All experiments were done in a microtiterplate and the OD405 was scanned every 5 minutes for 6h.

**Example 6: Certain anti FIX/FIXa-antibodies are procoagulant in the presence of FIXa**

During normal hemostasis, FIX becomes initially activated either by the tissue factor (TF)/factor VIIa pathway or later on by activated factor XI (FXIa). Subsequently to its activation, FIXa associates on the platelet surface in a membrane bound complex with activated FVIII. Factor IXa by itself has little or no enzymatic activity towards FX, but becomes highly active in the presence of FVIIIa. To demonstrate that certain anti FIX/FIXa-antibodies have FVIII-like activity and hence are procoagulant in a FVIII deficient human plasma, the following experiment was carried out: Different amounts of antibody 193/AD3 or as a control mouse IgG were used in a standard APTT based one stage clotting assay. Briefly, 100µl antibody containing samples were incubated with 100µl of FVIII deficient plasma (DP) and with 100µl of DAPTTIN (PTT Reagent for determining activated Thromboplastin Time; IMMUNO AG) reagent, in a KC10A clotting analyzer. Where indicated, a total amount of 50ng activated FIX was included into the reaction mixture. After 4 minutes incubation, the reaction was started by the addition of 100µl  $\text{CaCl}_2$  (25mM). The results are shown in table 1 and Fig. 9.

025791

µg AB	clotting time (sec)	
	193/AD3	mouse IgG
	50ng FIXa	50ng FIXa
9	101,6	102,5
4,5	95,6	103,2
2,25	93,1	103,2
1,8	93,7	101,9
1,35	91,4	103,4
0,9	94,4	102,2
0,45	98,1	101,9
0,34	97,1	103,9
0,23	99,3	103,7

Table 1: Clotting times of FVIII deficient plasma in an APTT based clotting assay employing various amounts of procoagulant (193/AD3) and control antibody (mouse IgG) in the presence of 50ng activated FIX (0.01U FIX). The molar ratio of antibody in the reaction and activated FIX is 10:1. The molar ratio between antibody and total FIX (FIX and FIXa, assuming that human FVIII deficient plasma contains 1U (5µg) FIX varies between 6:1 (9µg antibody in reaction) to 1:6 (0,23µg antibody in reaction). At the optimal shortening of the clotting time the molar ratio between antibody and total FIX is 1:1. The clotting time without the addition of FIXa is in the range of 120 seconds.

Fig. 9 shows a graphical representation of the clotting times of FVIII deficient plasma in an APTT based clotting assay employing various amounts of procoagulant (193/AD3) and control (mouse IgG) antibody in the presence of 50ng activated FIX. There is a clear (and reproducible) dose dependent reduction of the clotting time in samples supplemented with antibody 193/AD3. These results imply that antibody 193/AD3 is procoagulant in the presence of FIXa.

**Example 7: Anti FIX/FIXa-antibodies are procoagulant in the presence of FVIII inhibitors and FIXa**

A severe complication of the standard FVIII substitution therapy is the development of alloantibodies directed against FVIII,

05791

leading to FVIII neutralization and a condition where the patients blood becomes inclottable.

To demonstrate that certain anti FIXa-antibodies have FVIII-like activity even in the presence of FVIII inhibitors, the following experiment was carried out: Different amounts of antibody 193/AD3 or as a control mouse IgG were used in a standard APTT based one stage clotting assay. Briefly, 100µl antibody sample were incubated with either 100µl of FVIII deficient plasma (Fig.10A) or FVIII inhibitor plasma (inhibitor potency 400BU/ml), Fig.10B) as well as with 100µl of DAPTTIN reagent, in a KC10A clotting analyzer. In addition, a total amount of 50ng activated FIXa was included into the reaction mixture. After 4 minutes incubation, the reaction was started by the addition of 100µl CaCl<sub>2</sub> (25mM). To ensure equal conditions, the experiment employing FVIII deficient plasma and FVIII inhibitor plasma were done side by side. The results are shown in Fig. 10A and 10B. As already shown in Example 6, there is a clear (and reproducible) dose dependent reduction of the clotting time in samples supplemented with antibody 193/AD3 have in the presence of FVIII inhibitors.

**Example 8: Anti FIXa-antibodies are procoagulant in the presence of defective FVIII and FIXa**

To demonstrate that certain anti FIXa-antibodies have FVIII-like activity in the presence of defective FVIII, the following experiment might be carried out: Increasing amounts of antibody 193/AD3 or as a control mouse IgG are used in a standard APTT based one stage clotting assay. In this clotting assay, hemophilia A patients plasma having very low clotting activity, due to the presence of defective FVIII (DF8) is used. Briefly, 100µl antibody sample are incubated with either 100µl of DF8 plasma or FVIII deficient plasma as well as with 100µl of DAPTTIN reagent, in a KC10A clotting analyzer. In addition, a total amount of 50ng activated FIXa will be included into the reaction mixture. After a short incubation, the reaction will be started by the addition of 100µl CaCl<sub>2</sub> (25mM). To ensure equal conditions, the experiment employing FVIII deficient plasma and DF8 plasma is done site by site.

025791

**Example 9: Anti FIX/FIXa-antibodies with procoagulant activity in the presence of FIXa distinguish between human and bovine FIXa**

FIX/FIXa specific monoclonal antibodies selected from the 198<sup>th</sup> fusion experiment were purified from the respective hybridoma supernatant and quantified as described in example 3. These antibodies were analyzed in a modified one stage clotting assay (as described in Example 6) and some showed procoagulant activity.

The chromogenic activity of these antibody preparations was measured in the following FXa generation kinetic assay: 10µg of monoclonal antibody (in 25µl) were transferred to microtiter plate wells and warmed to 37°C. Chromogenic substrate (S-2222), synthetic thrombin inhibitor (I-2581), factor (F) IXa and FX were reconstituted in sterile water and FIXa/FX (both bovine) mixed with phospholipids according to the suppliers protocol. Per reaction, 50µl of the phospholipid/FIXa/FX solution were combined with 25µl CaCl<sub>2</sub> (25mM) and 50µl of the substrate/inhibitor cocktail. To start the reaction, 125µl of the premix were added to the monoclonal antibody solution in the microtiter plates and incubated at 37°C. Absorbancy at 405nm and 490nm of the samples was read at various times (5min to 2h) against a reagent blank (25µl TBS instead of monoclonal antibodies) in a Labsystems iEMS Reader MF<sup>TM</sup> microtiter plate reader using GENESIS<sup>TM</sup> software. In parallel, the same reactions were performed except that 50ng human FIXa were added per reaction. Those antibodies that showed procoagulant activity had no chromogenic activity in the case of bovine FIX, but displayed high activity when human FIXa was present.

Fig. 11 shows the time course of the chromogenic FVIII like activity exhibited by the monoclonal antibodies 198/A1, 198/B1 and 198/AP1 with (+) and without (-) addition of 50ng human FIXaβ. Non-specific polyclonal mouse IgG was used as a control. 198/A1 and 198/B1 show procoagulant activity (similar as 193/AD3 in example 6) whereas 198/AP1 does not. Antibody 198/BB1 had the same activity pattern (data not shown).

05791

**Example 10 : Structure and procoagulant activity of antibody derivatives derived from anti FIX/FIXa-antibodies**

**Subcloning antibody variable domains from hybridoma cell lines 193/AD3, 193/K2 and 198/B1:**

**Cloning procedure:** Messenger RNA was prepared from  $1 \times 10^6$  hybridoma cells of the respective cell line (either 193/AD3, 193/K2 or 198/B1) employing the "QickPrep® Micro mRNA Purification Kit" (Pharmacia) according to the manufacturers instruction. The corresponding cDNA was produced by retro transcription of mRNA using the "Ready-To-Go-You-Prime-First-strand Beads kit" (Pharmacia) according to the manufacturers instructions. Heavy and light chain encoding sequences were converted to the corresponding cDNA employing a set of primers. To reverse transcribe heavy chain specific mRNA (VH) an equimolar mixture of the oligonucleotides MOCG1-2FOR (5' CTC AAT TTT CTT GTC CAC CTT GGT GC 3'), MOCG3FOR (5' CTC GAT TCT CTT GAT CAA CTC AGT CT 3') and MOCMFOR (5' TGG AAT GGG CAC ATG CAG ATC TCT 3') was used (RTmix1). In the same reaction tube, light chain specific cDNA (VL) was synthesized using primer MOCKFOR (5' CTC ATT CCT GTT GAA GCT CTT GAC 3').

The coding sequences for VH were amplified by PCR using the primer-sets depicted in Fig. 12 and the specific cDNA, derived from the reverse transcription mixture (RTmix1) described above, as template. VK-chain genes were amplified using the primer sets depicted in Fig. 13 and employing also RTmix1 as a template. The VH-PCR product was cleaved SfiI-AscI and inserted into SfiI-AscI digested vector pDAP2 (GeneBank accession no.: U35316). The

---

obtained pDAP2-VH constructs, were named pDAP2-193AD3/VH and pDAP2-193/K2/VH, respectively. Both plasmids were subsequently cleaved with AscI-NotI and the corresponding AscI-NotI digested VK-gene PCR product was inserted. The resultant vectors were designated pDAP2-193/AD3scFv and pDAP2-193/K2scFv and code for the VH-gene and the VL-gene of the monoclonal antibodies 193/AD3 and 193/K2. Both chains are linked by the coding sequence for a artificial, flexible linker (G<sub>4</sub>SGGRASG<sub>4</sub>S; Engelhardt et al, 1994)



05791

and enables expression of the scFv variant of the respective antibody.

In Fig. 14, the DNA and the deduced protein sequence of the scFv derived from the hybridoma cell line 193/AD3 is depicted. Nucleotides 1 to 357 code for the heavy chain variable domain, nucleotides 358 to 402 code for the artificial flexible linker and nucleotides 403 to 726 code for the light chain variable region. The protein sequence of the CDR3 region of the heavy chain has the sequence YGNSPKGFAY and is given in bold letters. The artificial linker sequence (G<sub>4</sub>SGGRASG<sub>4</sub>S) is shown in italics.

In Fig. 15, the DNA and the deduced protein sequence of the scFv derived from the hybridoma cell line 193/K2 is shown. Nucleotides 1 to 363 code for the heavy chain variable domain, nucleotides 364 to 408 code for the artificial flexible linker and nucleotides 409 to 747 code for the light chain variable region. The protein sequence of the CDR3 of the heavy chain has the sequence DGGHGYGSSFDY, and is given in bold letters. The artificial linker sequence (G<sub>4</sub>SGGRASG<sub>4</sub>S) is shown in italics.

In Fig. 16, the DNA and the deduced protein sequence of the scFv derived from the hybridoma cell line 198/B1/1 is depicted. Nucleotides 1 to 366 code for the heavy chain variable domain, nucleotides 367 to 411 code for the artificial flexible linker and nucleotides 412-747 code for the light chain variable region. The protein sequence of the CDR3 region of the heavy chain has the sequence EGGGFTVNWYFDV and is given in bold letters. The artificial linker sequence (G<sub>4</sub>SGGRASG<sub>4</sub>S) is shown in italics.

---

The derivatives are tested and show procoagulant activity in the chromogenic assay.

**Example 11: Procoagulant activity of peptides derived from CDR3 regions of anti FIX/FIXa-antibodies**

The antigen binding site of an antibody is derived from the juxtaposition of the six "complement determining regions (CDR's)" at the N-terminal end of the VL-HL dimer (or Fv

025791

region). Three CDR's are derived from the light chain and three are derived from the heavy chain. The contribution of a singular CDR to the antibody specificity for a given antigen may vary considerably, but in general it is thought that the CDR3 region of the heavy chain (CDR3<sub>H</sub>) is of special influence i.e. the particular protein sequence of CDR3<sub>H</sub> region may be highly important for the antigen recognition. The length of CDR3<sub>H</sub> regions has been reported to vary considerably and is in the range of 4-25 amino acids (Borrebaeck, p.16).

FVIII derived peptides show some procoagulant activity in an in vitro assay. For a single hemophilia patients plasma, a reduction of the APTT based clotting time of 20 seconds compared to baseline could be demonstrated (Liles et al., 1997). In a similar experiment we use the peptides derived from the CDR3<sub>H</sub> region (the putative FIXa interacting region) of antibodies 193/K2, 193/AD3 and 198/B1 to demonstrate a dose dependent reduction of the clotting time of a FVIII deficient plasma as compared to a control peptide.

025791

peptide source	peptide sequence	remarks
193/AD3	YGNSPKGFAY	CDR3H 193/AD3#6
	GANGYKYPFS	CDR3H 193/AD3#6 scrambled version (control)
	CXXYGNSPKGFAYXXC	CDR3H 193/AD3#6 cyclic peptide 1
	YGNSPKGFAY	CDR3H 193/AD3#6 cyclic peptide 2
193K2#1	DGGHGYGSSFDY	CDR3H 193K2#1
	SGHDGSYFYGDG	CDR3H 193K2#1 scrambled version (control)
198B1	EGGGFTVNWYFDV	CDR3H 198B1
	GFVWGVEDGYTF	CDR3H 198B1 scrambled version (control)
hFVIII	FRNRGMTALLKVSSCD	FVIII 697-712 (Liles et al., 1997)
	SVKLFGNMSDRLARCT	FVIII 697-712 scrambled version (control)

Table 2: Peptides used in an one-stage APTT based clotting assay. X can be any amino acid.

**Example 12: Procoagulant activity of (peptide) derivatives obtained from CDR3 regions of anti FIX/FIXa-antibodies**

In principle, the antibody molecule can be envisioned as a biological device for the presentation of a combinatorial array of peptide elements in three dimensional space (see Gao et al., 1999, PNAS, 96:6025). Therefore, an antibody (or an antibody derivative i.e. scFv, Fab etc) can be used either as a tool for the detection of functional important domains of a specific target protein and on the other hand, for the delineation of amino acid sequences specifically mediating certain interactions, i.e. activating or enhancing the activity of FIXa

025791

towards the physiological substrate FX. The latter process has lead to the evaluation of a number of CDR3H derived peptide sequences as FIXa enhancing agents (example 11). On the other hand, the procoagulant activity of the peptides obtained so far is not optimal but is further improved by sequence variation within the peptide regions critical for mediating the FIXa activity enhancing region.

As a first step towards peptide sequences with enhanced procoagulant activity, the binding site of antibody 198/B1 at the FIXa molecule is mapped employing sequence comparison analysis, competitive binding assays, western blot analysis and competitive ELISA analysis. Since the crystal structure of FIX is known, molecular modeling is used to improve the fitting of 198/B1 derived peptides in the 198/B1 binding site on human FIXa. The data so obtained will allow us to do a methodical mutational analysis of the peptide CDR3H 198/B1. Briefly, peptides (or peptide libraries in case of a high throughput screening approach) containing specific mutations (rational for a specific mutation is given i.e. by molecular modeling) will be assayed for procoagulant activity.

A example of a methodical mutational analysis of the wildtype CDR3H 198/B1 amino acid sequence (EGGGFTVNWYFDV) is given below. The peptide sequence was varied only in one position across the core sequence (changes are shown in bold letters). All the peptides can be assayed for procoagulant activity in a APTT based one stage clotting assay and used at the same concentration. Clotting activity of the wildtype sequence is arbitrarily set as 100%.

---

peptide sequence

EGGGFTVNWYFDV

EGGGRTVNWYFDV

EGGGFRVNWYFDV

EGGGFTRNWYFDV

EGGGFTVRWYFDV

EGGGFTVNRYFDV

025791

EGGGFTVNWRFDV  
EGGGFTVNWYRDV

EGGGATVNWYFDV  
EGGGFAVNWYFDV  
EGGGFTANWYFDV  
EGGGFTVAWYFDV  
EGGGFTVNAYFDV  
EGGGFTVNWAFDV  
EGGGFTVNWYADV

EGGGDTVNWYFDV  
EGGGFDVNWYFDV  
EGGGFTDNWYFDV  
EGGGFTVDWYFDV  
EGGGFTVNDYFDV  
EGGGFTVNWDFDV  
EGGGFTVNWYDDV

EGGGPTVNWYFDV  
EGGGFPVNWYFDV  
EGGGFTPNWYFDV  
EGGGFTVPWYFDV  
EGGGFTVNPYFDV  
EGGGFTVNWPFDV  
EGGGFTVNWYPDV

Table 3: Generation of 198/B1 derived peptide variants.

**Example 13: Conversion of the 196/C4 IgM into IgG1:**

---

Since some IgM antibodies demonstrate an extremely high chromogenic FVIII-like activity, attempts were made to convert IgM antibodies in IgG (but also in a recombinant Fab, F(ab)<sub>2</sub>, scFv etc). Described in detail is the rescue of the IgM variable region genes:

Construction of the expression plasmid pBax-IgG:

025791

Vector pBax-IgG1 (Fig. 17) was constructed from vectors pSI (Promega) and pEF/Bsd (Invitrogen) through multiple cloning steps.

B-lymphocytes of a donor are purified from blood and mature mRNA was purified from these cells using the "micro-mRNA-purification-kit" (Pharmacia). The cDNA of a human kappa chain and a human gamma 1 chain are prepared employing the "you-prime-first-strand-cDNA-kit" (Pharmacia) and the following primers:

The coding sequence of a human kappa light chain constant domain is amplified from the cDNA by PCR using specific primers:

The gene of a human gamma 1 chain constant region (CH1-hinge-CH2-CH3) is amplified from the cDNA by PCR using specific primers:

The PCR product of the light chain constant domain is digested with and inserted into digested pSI. The resultant vector is cleaved with and the following annealed oligonucleotides were inserted resulting in vector pSI-Ckappa. The annealed oligonucleotides provide for the leader and the SacI-XbaI sites for insertion of the kappa chain variable region. The PCR product of the human gamma 1 chain constant region is digested with and inserted into digested pSI. The resultant vector is cleaved with and the following annealed oligonucleotides are inserted resulting in vector pSI-Cgamma. The annealed oligonucleotides provide for the leader and the XhoI-BstEI sites for insertion of the heavy chain variable region. Vector pEF/Bd is digested NheI and SfiI, blunt ended by Klenow treatment and the whole expression cassette of pSI-Ckappa, excised with BglII and BamHI is inserted (after Klenow treatment). The resultant vector is digested EcoRI and HindIII and treated with Klenow. The whole expression cassette of pSI-Cgamma is excised with BglII and BamHI is inserted (after Klenow treatment). The resultant vector is named pBax-IgG1.

The light chain variable region can be inserted in between the SacI-XbaI sites, yielding the complete coding-sequence of a kappa light chain. The heavy chain variable region can be cloned

05791

in between the XhoI-BstEI sites, resulting in a complete IgG1 heavy chain gene. Both open reading frames are expressed under the control of the SV40-promoter and harbour the coding sequence of a signal peptide at the 5' end of the genes for secretion of the heavy and light chains into the endoplasmatic reticulum. Transfection into COS cells allows the expression of an IgG1 with the same binding properties as the parental IgM.

#### Construction of the plasmid pBax-196/C4

The VH of the 196/C4 scFv (subcloned as described in Experiment) is amplified by PCR using the primers. The PCR product is digested XhoI and BstEII and inserted into XhoI and BstEII digested pBax IgG1. The VL of the 196/C4 scFv is amplified by PCR using the primers. The PCR product is digested SacI and XbaI and inserted into SacI and XbaI digested pBax IgG1-VH. The resultant vector (pBax-196/C4) is transfected into COS cells by electroporation and hybrid IgG1 molecules (murine variable region and human constant region) with the same specificity as the parental IgM is expressed.

025791

## Claims :

1. Antibody or antibody derivative against factor IX/factor FIXa, characterized in that it reinforces the procoagulant activity of the FIXa.

5 2. Antibody or antibody derivative against FIX/FIXa, characterized in that it comprises factor FVIIIa (FVIIIa)-like cofactor activity.

3. Antibody or antibody derivative against FIX/FIXa, characterized in that it reinforces the procoagulant activity of the FIXa, obtainable by a method comprising  
10 the steps:

- Immunization of an immunocompetent mouse with an antigen selected from the group consisting of FIX, FIXa $\alpha$ , FIXa $\beta$  or of fragments of them,
- Isolation of the spleen cells of the immunized mouse
- Production of hybridoma clones
- Screening of the hybridoma cell supernatants for reinforcement of the procoagulant activity of the factor IXa and selection of the sought antibodies or antibody derivative
- Isolation and purification of the antibodies or antibody derivatives.

4. The antibody or antibody derivative according to one of the Claims 1 to 3, characterized in that it reinforces the procoagulant activity of the FIXa in the presence of FVIII inhibitors.

15

5. The antibody according to one of Claims 1 to 4, characterized in that that it is from the class of IgG-, IgM-, IgA- or IgE antibodies.

6. The antibody or antibody derivative according to one of Claims 1 to 5,  
20 characterized in that that it was selected from the group consisting of monoclonal antibodies, antibody fragments, chimera antibodies, humanized antibodies, end



025791

chain antibodies, bispecific antibodies, diabodies and di-, oligo- or multimers of them.

7. The antibody derivative according to one of Claims 1 to 4, selected from the group  
5 consisting of peptidomimetica and peptides stemming from CDR.

8. The antibody derivative according to Claim 7, characterized in that it is a CDR3 peptide.

10 9. The antibody derivative according to Claim 8, characterized in that the CDR3 peptide contains an amino acid sequence selected from the group consisting of:

Tyr-Gly-Asn-Ser-Pro-Lys-Gly-Phe-Ala-Tyr or

Cys-X-X-Tyr-Gly-Asn-Ser-Pro-Lys-Gly-Phe-Ala-Tyr-X-X-Cys,

wherein X can be any amino acid, or

15 Tyr-Gly-Asn-Ser-Pro-Lys-Gly-Phe-Ala-Tyr or

Asp-Gly-Gly-His-Gly-Tyr-Gly-Ser-Ser-Phe-Asp-Tyr or

Phe-Arg-Asn-Arg-Gly-Met-Thr-Ala-Leu-Leu-Lys-Val-Ser-Ser-Cys-Asp.

10. The antibody or antibody derivative according to one of Claims 1 to 7,  
20 characterized in that the variable region contains the amino acids 1 to 357 and/or 403 to 726 according to fig. 14.

11. The antibody or antibody derivative according to Claim 10, characterized in that an artificial linker sequence is additionally contained.

25

12. The antibody or antibody derivative according to one of Claims 1 to 7, characterized in that the variable region contains the amino acids 1 to 363 and/or 409 to 747 according to fig. 15.

025791

13. The antibody or antibody derivative according to Claim 12, characterized in that an artificial linker sequence is additionally contained.

14. The antibody or antibody derivative according to one of Claims 1 to 7,  
5 characterized in that the variable region contains the amino acids 1 to 366 and/or 412 to 747 according to fig. 16.

15. The antibody or antibody derivative according to Claim 14, characterized in that an artificial linker sequence is additionally contained.

10

16. Hybridoma cell line which expresses an antibody or antibody derivative against factor IX/factor IXa according to one of Claims 1 to 15.

17. The hybridoma cell line according to Claim 16, characterized in that it is selected  
15 from the group of #196/AF1, #196/AF2, #193/AD3, #193/K2-1, #198/AC1/1, #198/AM1, #198/A1, #198/B1, #198AP1, 198/A1, 198/B1, 198/BB1 with the deposit numbers 99090924 (198/A1), 99090925 (198/B1), 99090926 (198/BB1).

20 18. An antibody or antibody derivative according to one of Claims 1 to 15, expressed by a hybridoma cell line according to one of Claims 14 or 15.

19. A DNA molecule, characterized in that it codes for an antibody or an antibody derivative against FIX/FIXa according to one of Claims 1 to 15.

25

20. A preparation containing an antibody or antibody derivative against FIX/FIXa according to one of Claims 1 to 15.

21. A preparation according to Claim 20, characterized in that FIXa $\alpha$  and/or FIXa $\beta$

025791

are additionally contained.

22. The use of a preparation according to one of Claims 20 or 18 for treating patients with blood clotting disturbances.

5

23. The use of a preparation according to one of Claims 18 to 22, characterized in that the blood clotting disturbances are selected from the group comprising hemophilia A, hemorrhagic diathesis.

10 24. The use of a preparation according to Claim 22, characterized in that the treatment of hemophilia inhibitor patients takes place.

25. The use of a preparation according to one of Claims 20 or 21, for the treatment of complications in anticoagulant therapies.

15

26. The use of the antibodies in antibody derivatives according to one of Claims 1 to 15 as detection agents for the identification of functional domains in target proteins.

20

025791

Abstract :

An antibody or antibody derivative against factor IX/activated factor IX (FIXa) which reinforces the procoagulant activity of the FIXa.

A1576/99-1

ORIGINAL TEXT

### Factor VIII-like activity in hybridoma cell supernatants

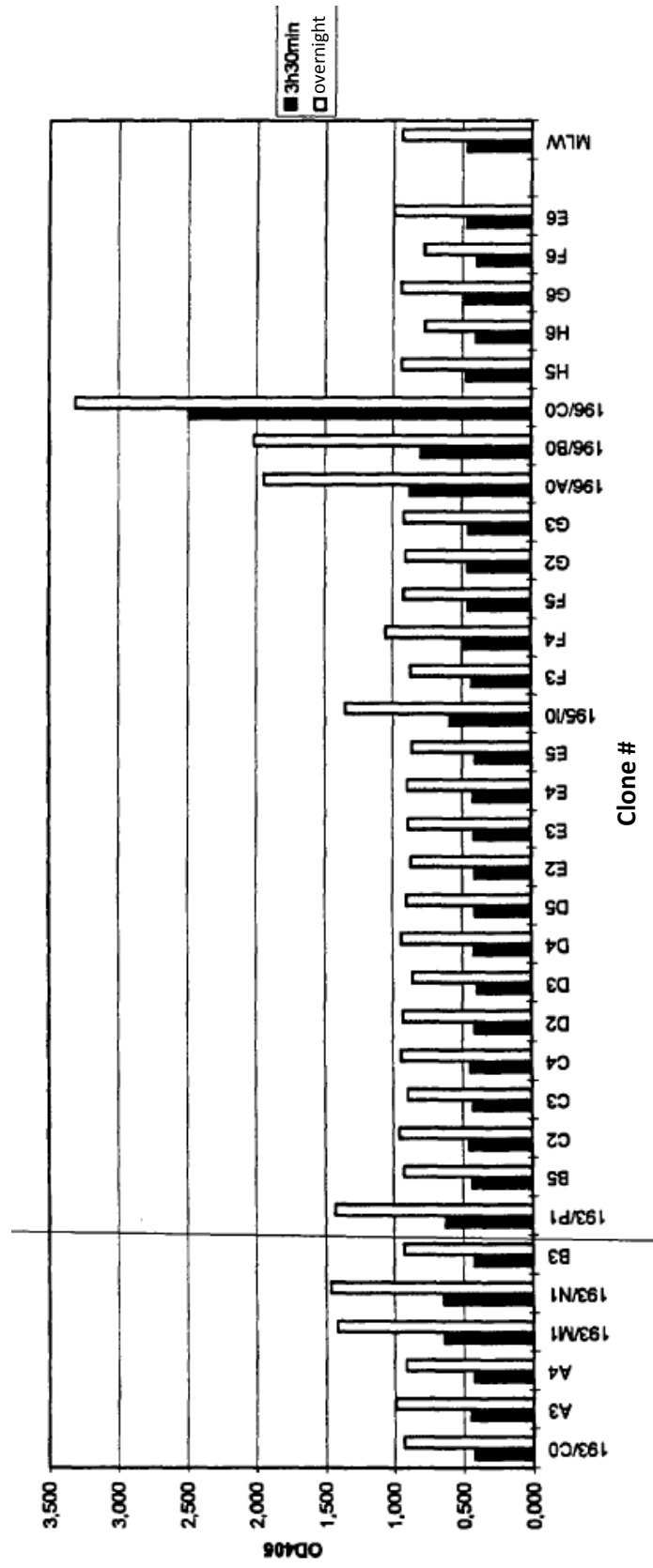


Fig. 1

00001

A1576/99-1

ORIGINAL TEXT

OD405

IgG mediated FVIII-similar activity in hybridoma cell supernatants of a master plate

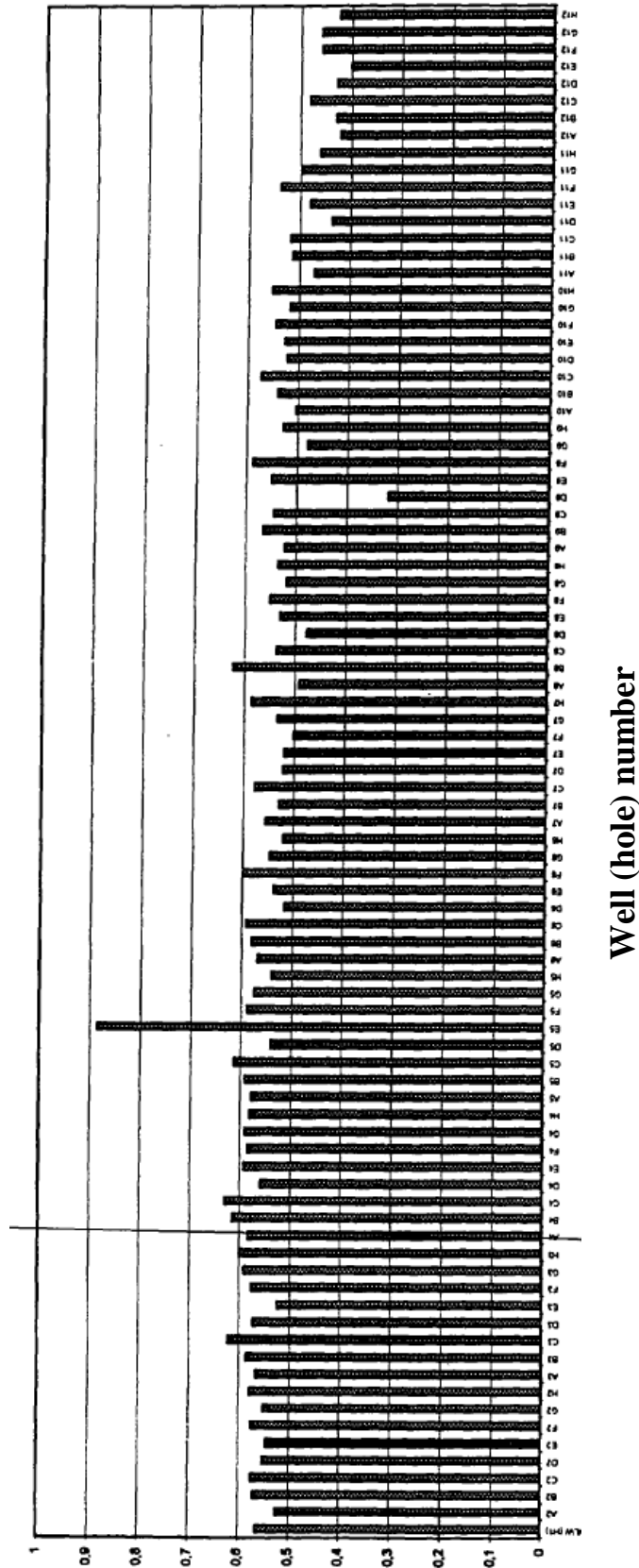


Fig. 2

A1576/99-1

ORIGINAL TEXT

OD405

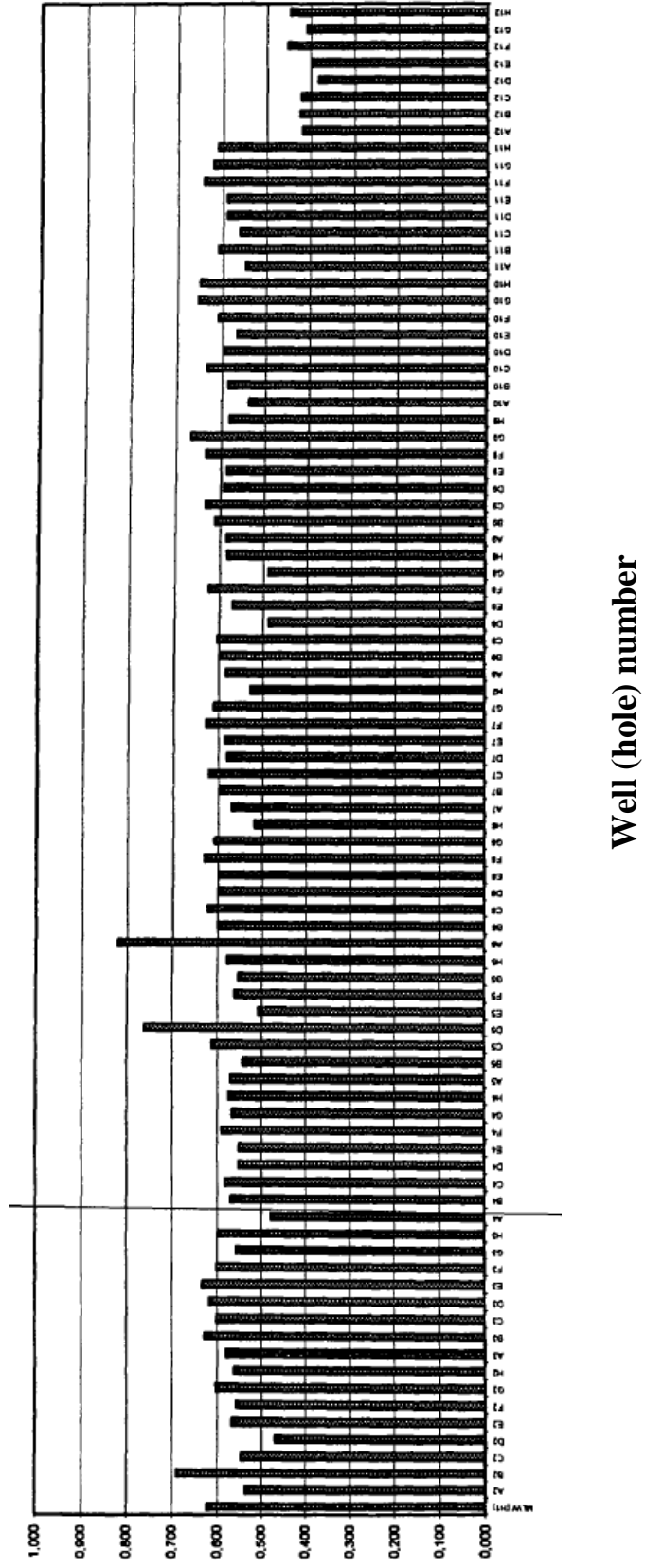


Fig. 3

A1576/99-1

ORIGINAL TEXT

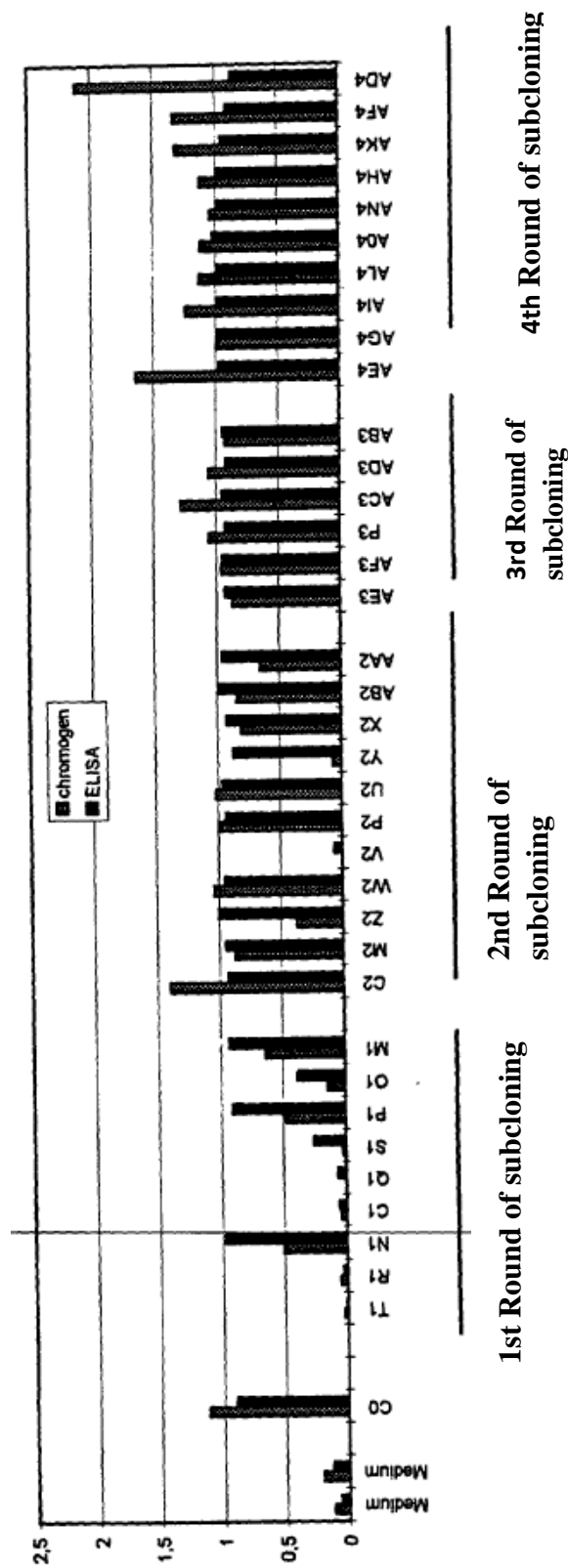


Fig. 4



00001

A1576/99-1

ORIGINAL TEXT

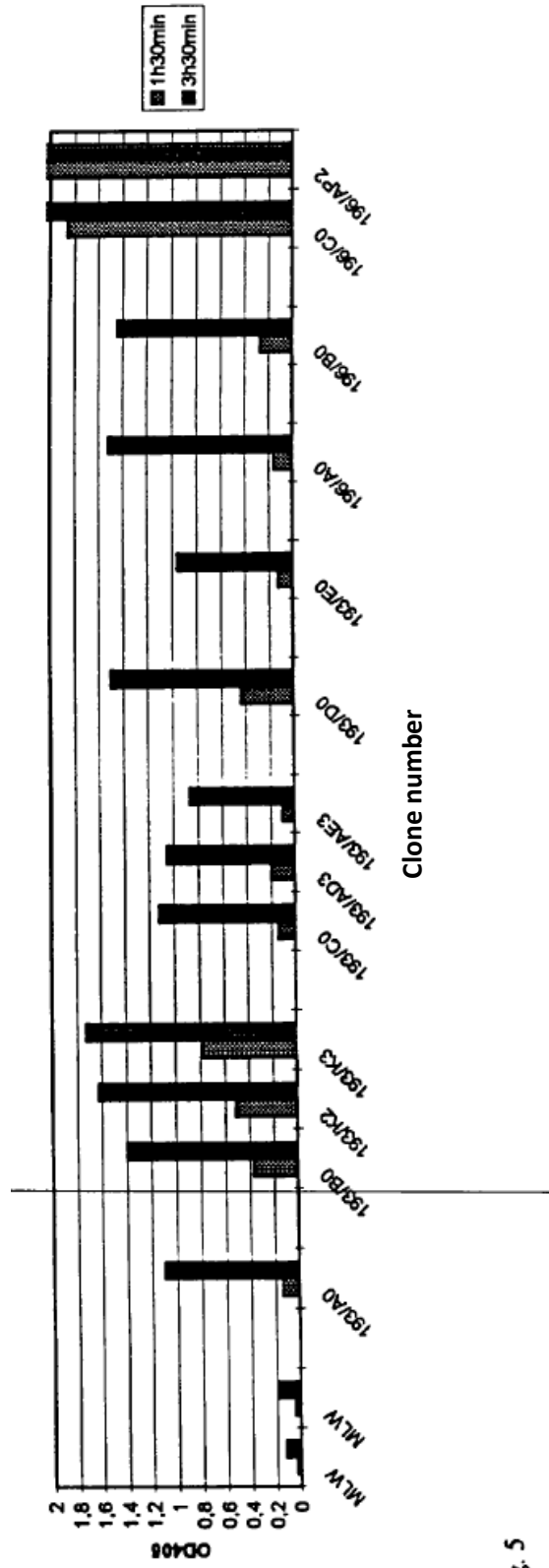


Fig. 5

A1576/99-1

ORIGINAL TEXT

FVIII-like activity of the antibodies 193/AD3 and 196/AF2

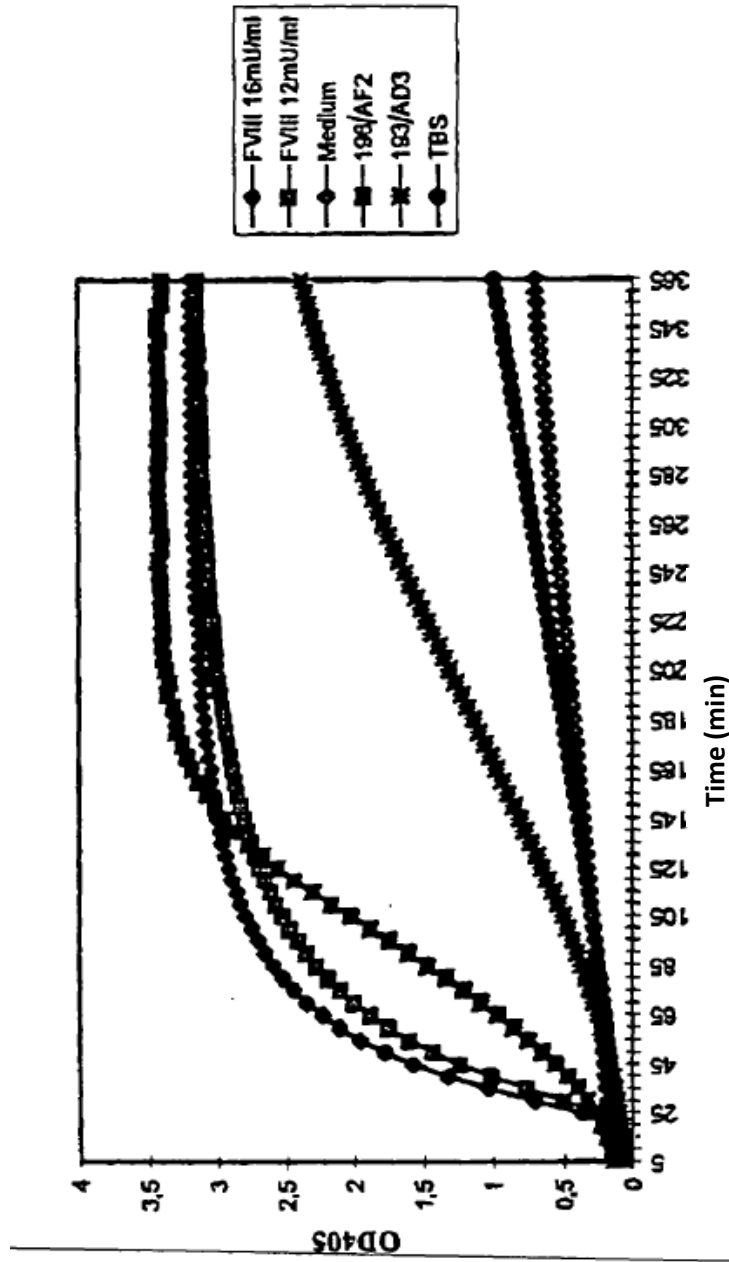


Fig. 6A

000001

A1576/99-1

ORIGINAL TEXT

Comparison of FVIII, 196/AF1, 198/AC1/1 and mouse IgG

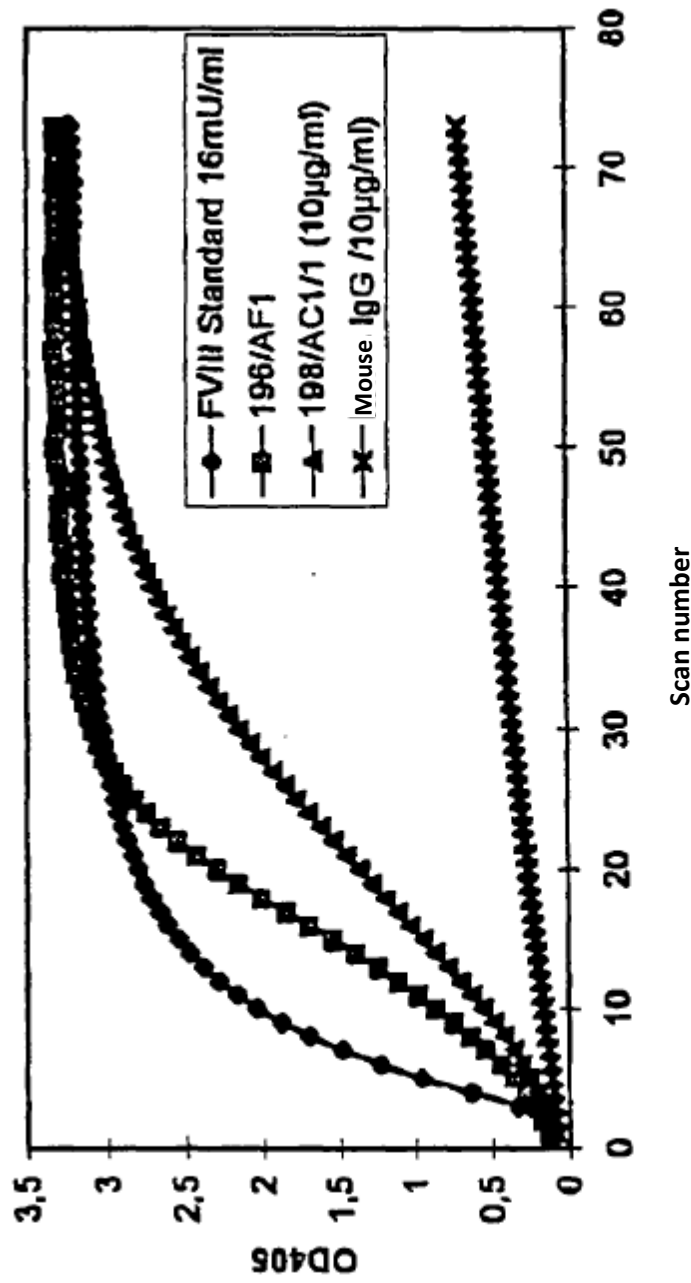
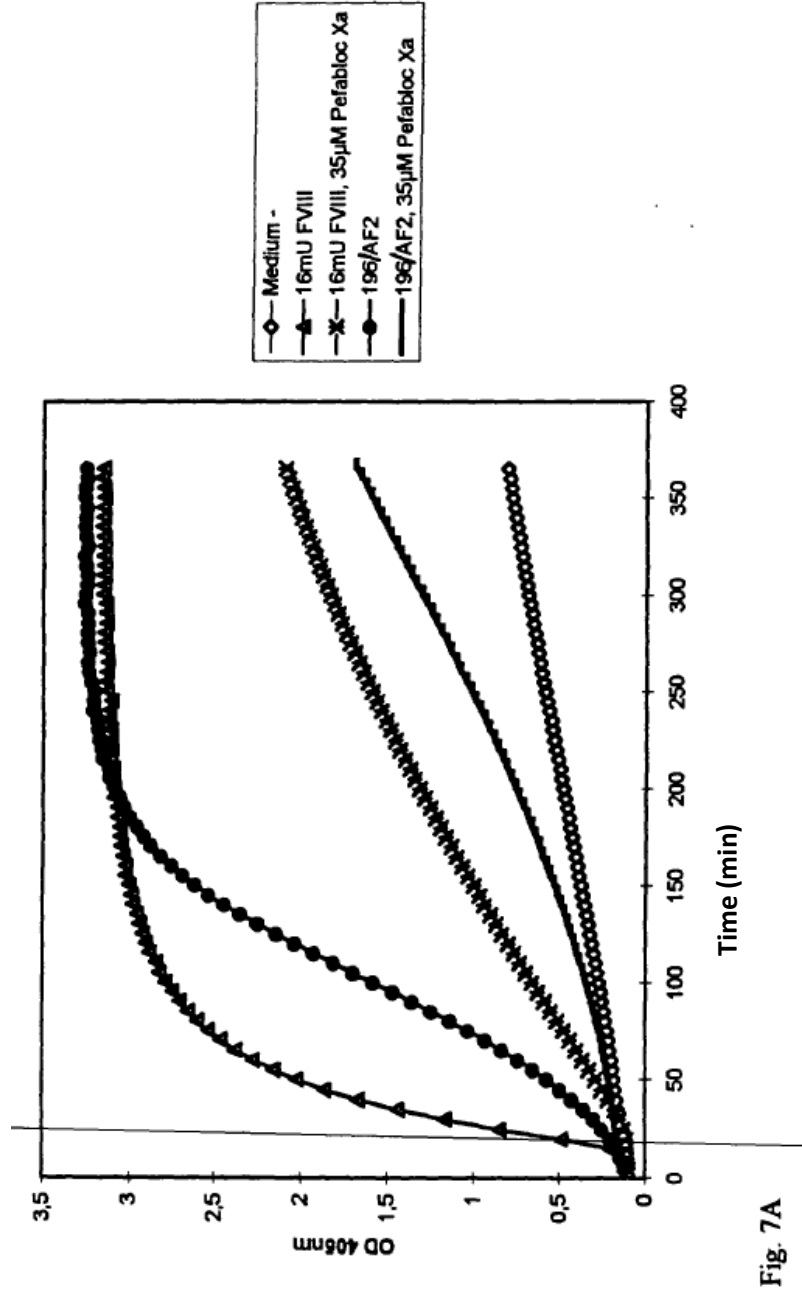


Fig. 6B

A1576/99-1

ORIGINAL TEXT

### Generation Kinetics of FXa



A1576/99-1

ORIGINAL TEXT

# Generation Kinetics of FXa

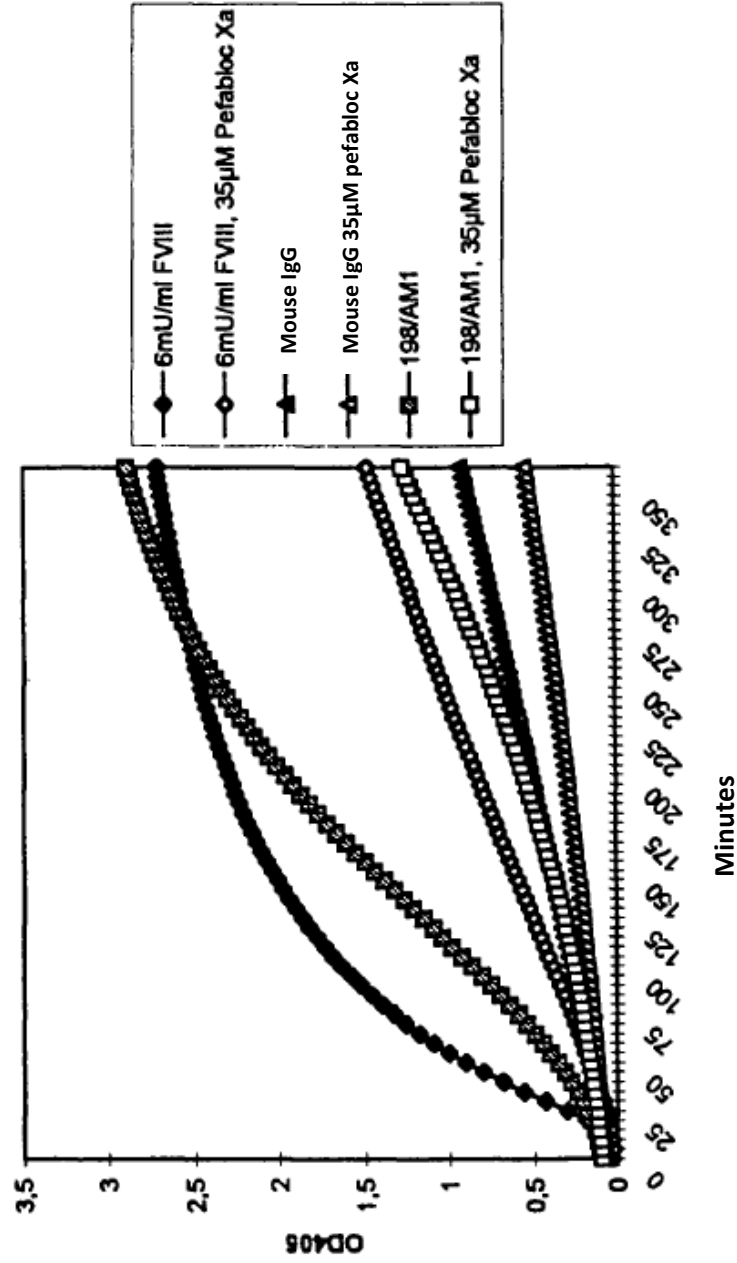


Fig. 7B

000001

A1576/99-1

ORIGINAL TEXT

Dependence of the 198/AC1/1 mediated FXa generation on the presence of PL, FIXa and Ca<sup>2+</sup>

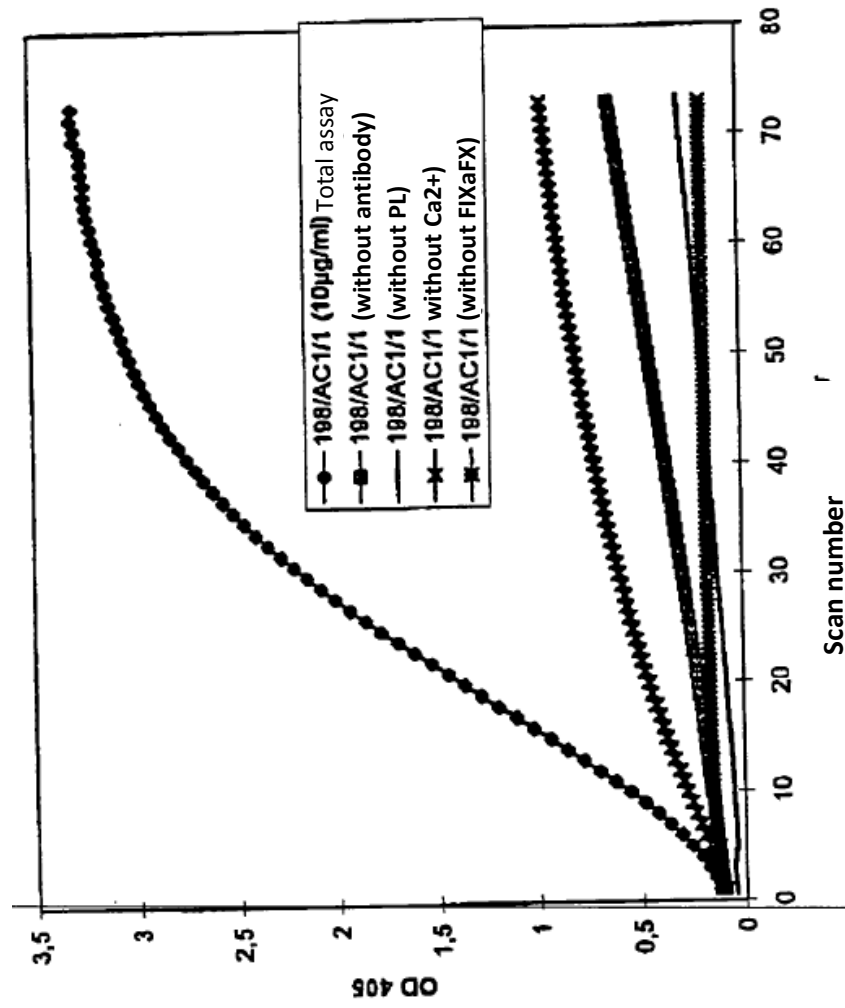


Fig. 8A

00001

A1576/99-1

ORIGINAL TEXT

Dependence of the 196/AF1 mediated FXa generation on the presence of PL, Ca<sup>2+</sup> and FIXa/FX

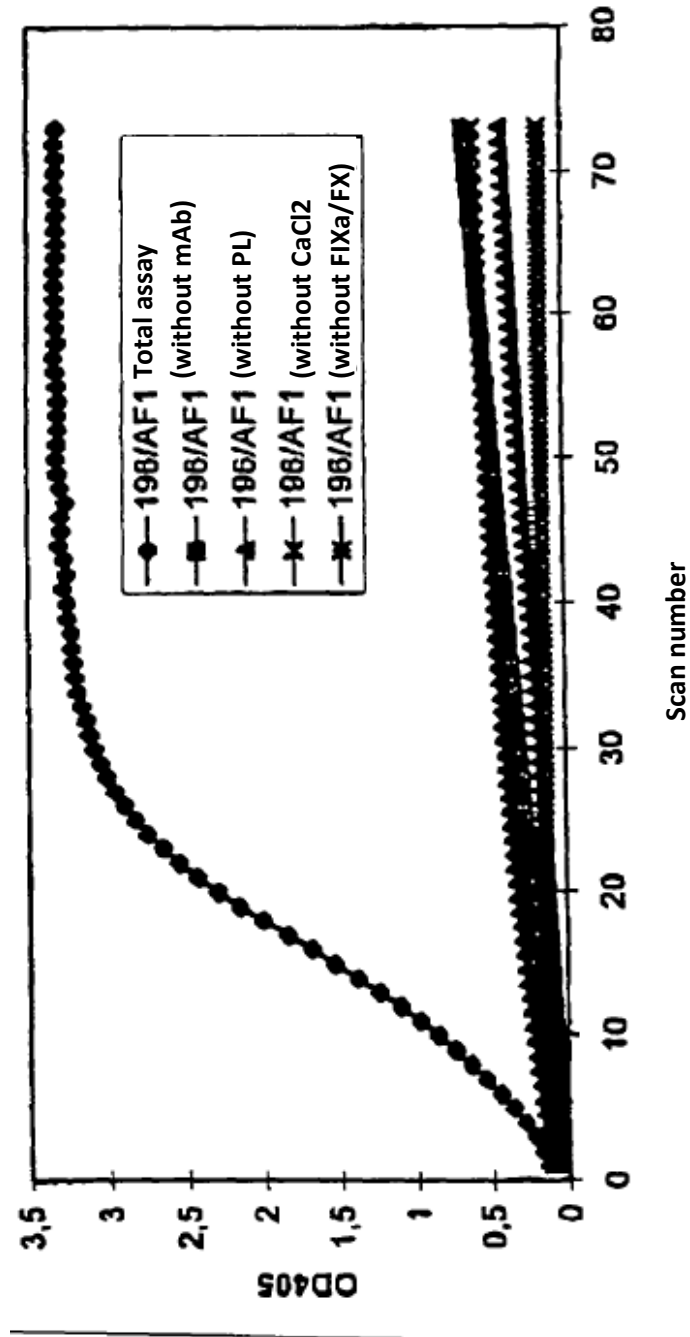


Fig. 8B

000001

A1576/99-1

ORIGINAL TEXT

# FXa generation by mouse IgG

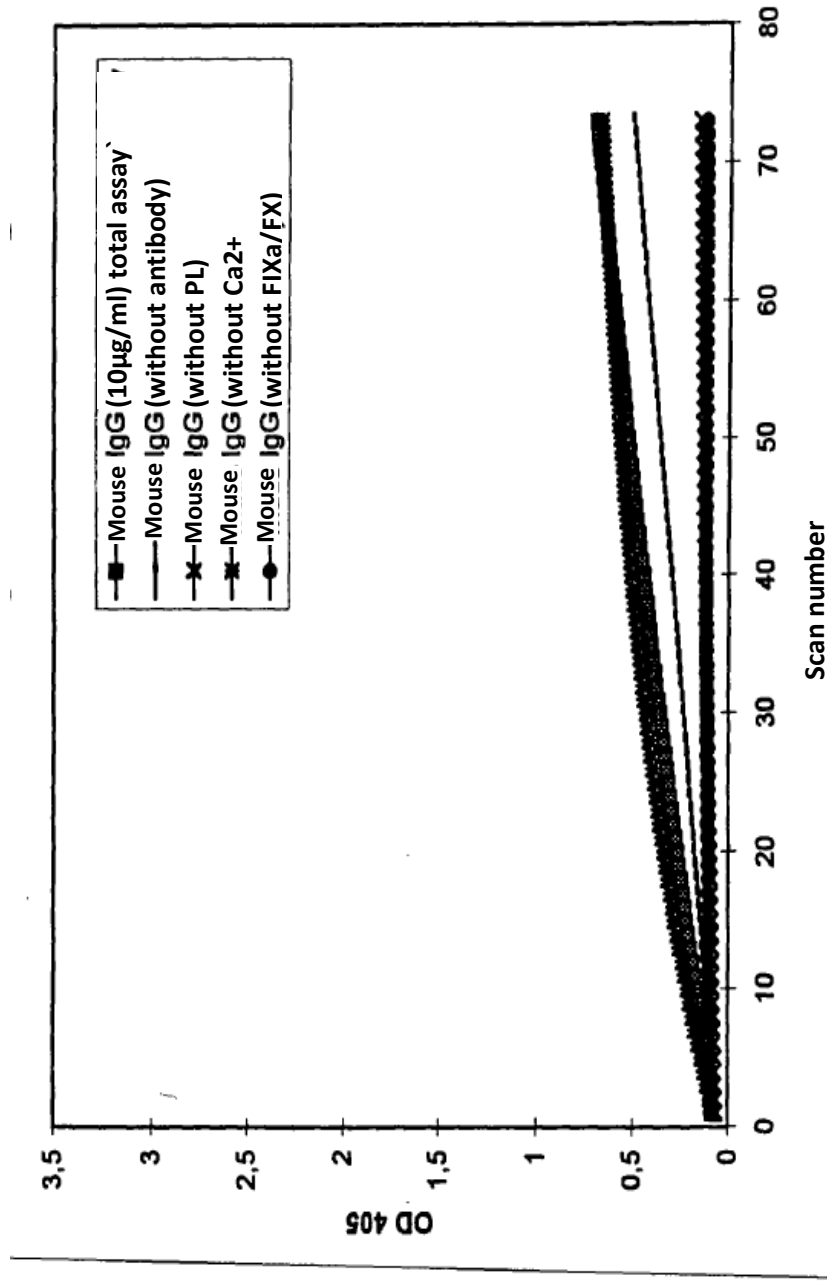


Fig. 8C



A1576/99-1

ORIGINAL TEXT

Clotting activity of the antibody 193/AD3 in the presence of FIXa

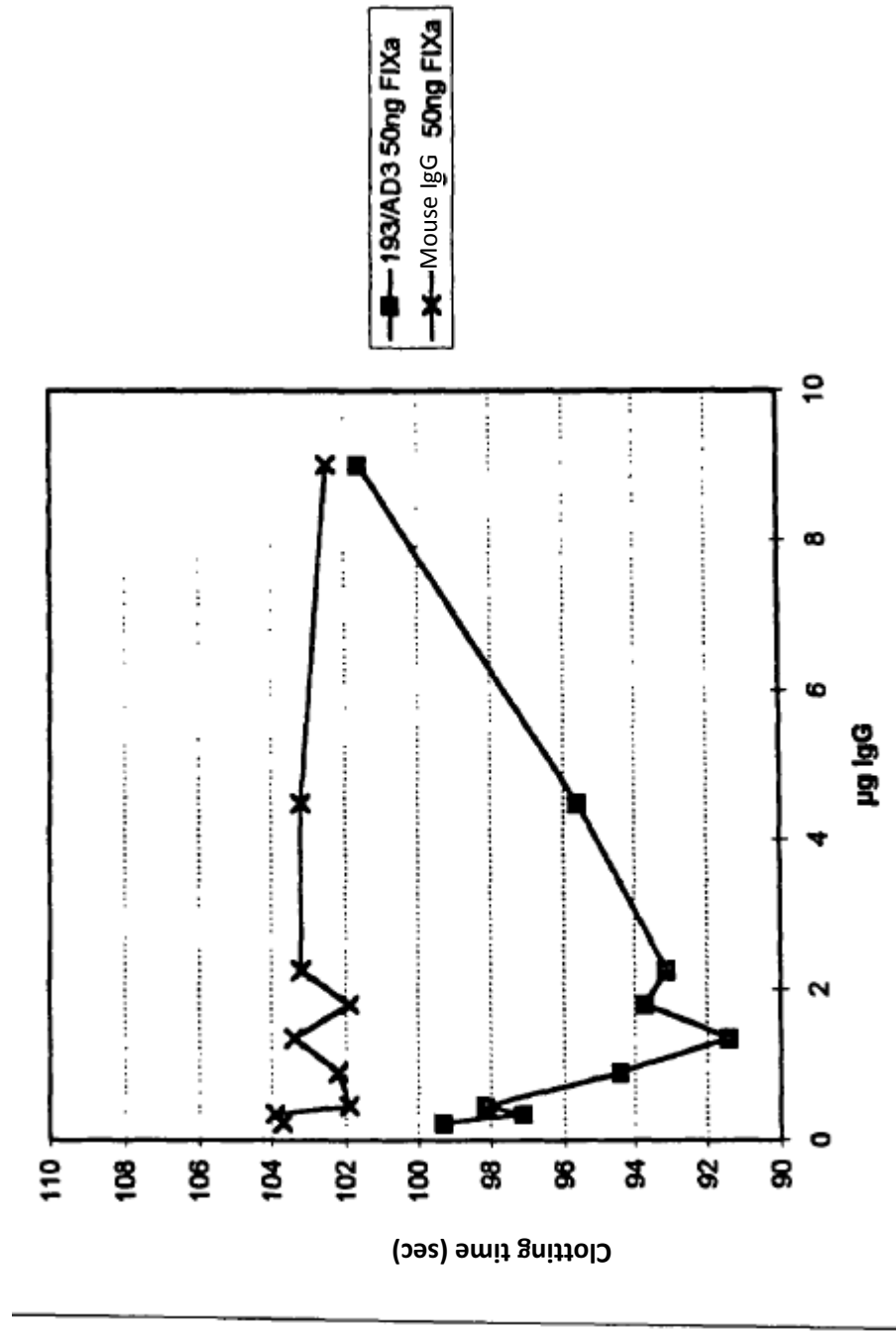


Fig. 9

00991

A1576/99-1

ORIGINAL TEXT

Procoagulant activity of the antibody 193/AD3 in FVIII deficient plasma in the presence of FIXa

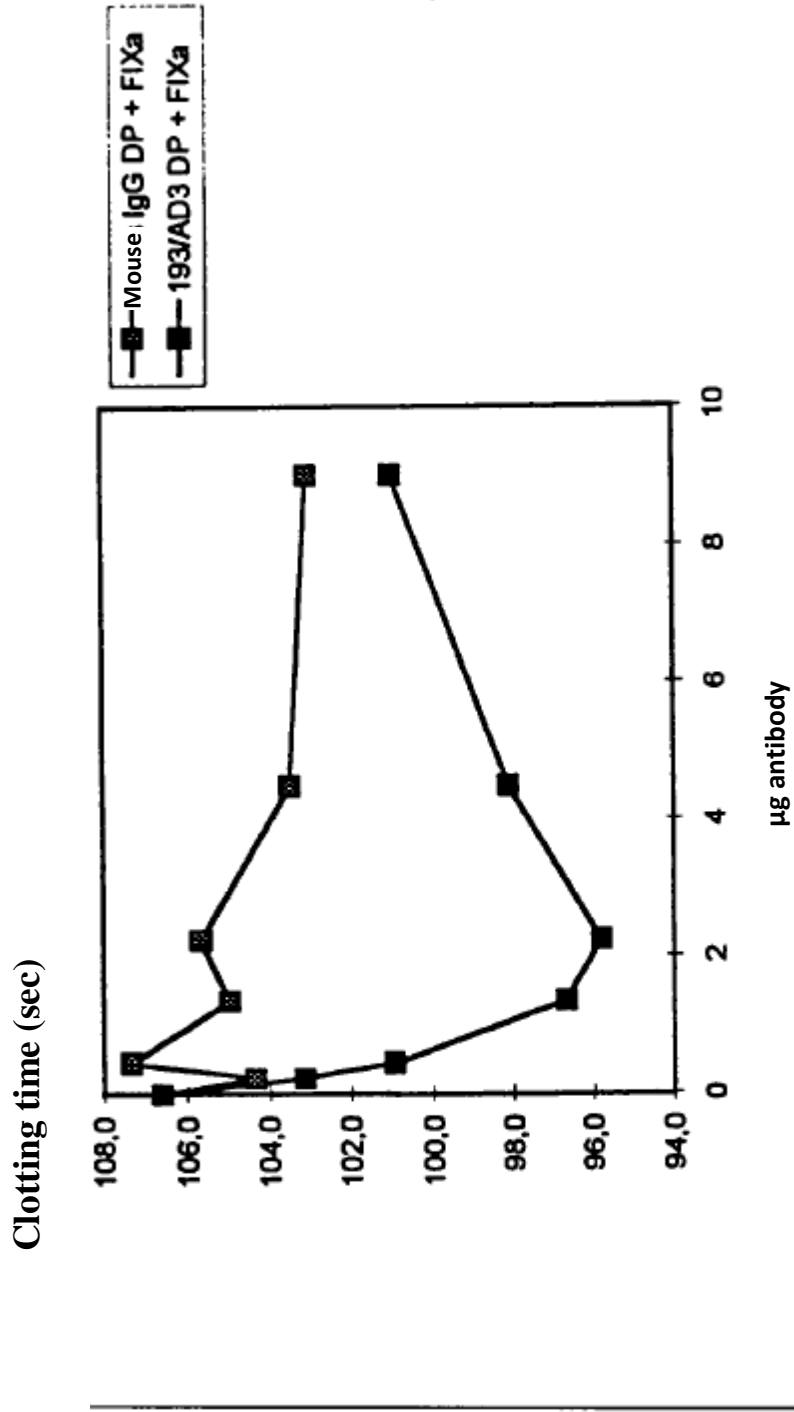


Fig. 10A

A1576/99-1

ORIGINAL TEXT

Procoagulant activity of the antibody 193/AD3 in FVIII deficient plasma in the presence of FIXa

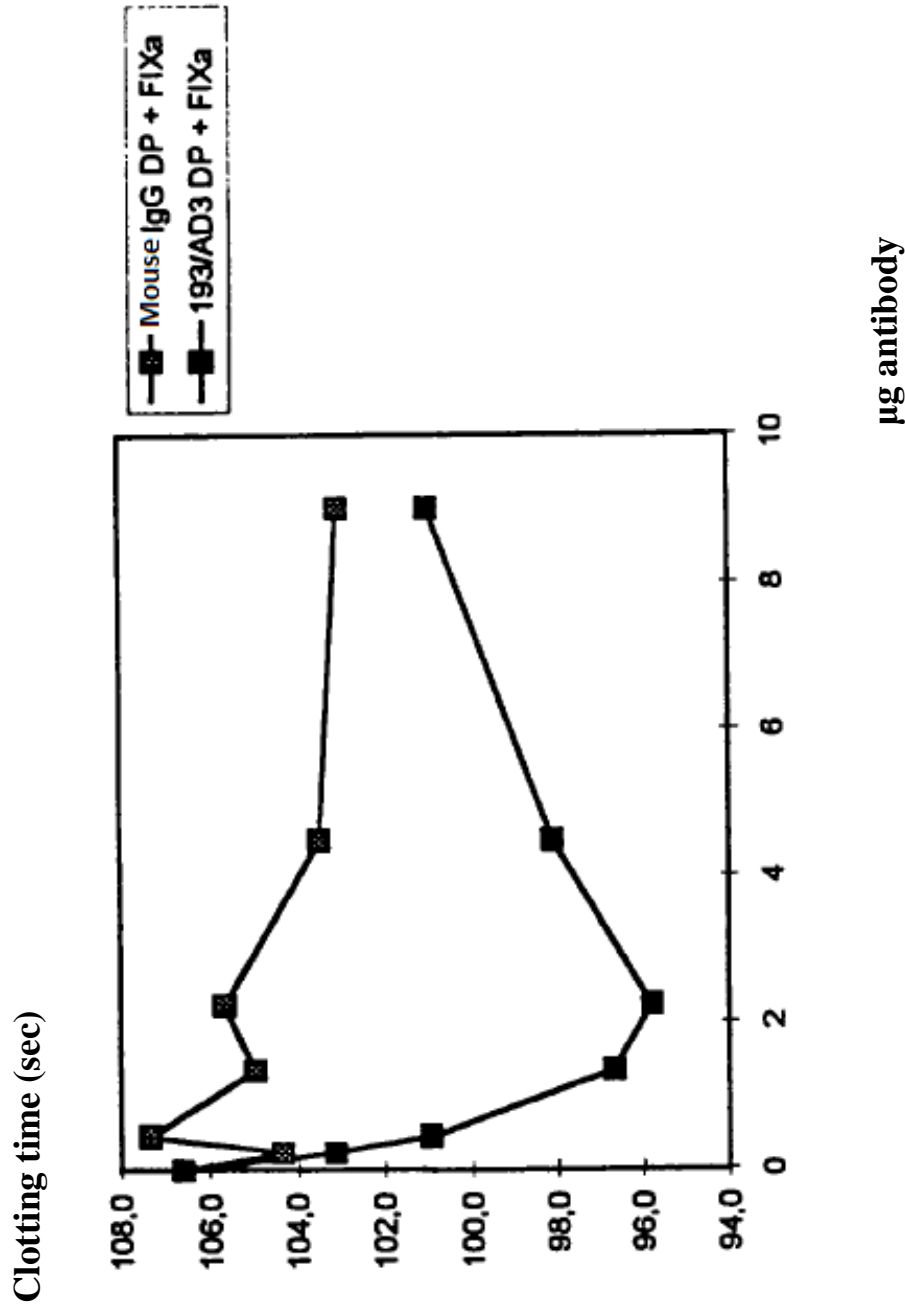


Fig. 10B

**A1576/99-1**

**ORIGINAL TEXT**

### Chromogenic activity of the antibodies 198/A1, 198/B1 and 198/AP1 the presence and the absence of human FIXa

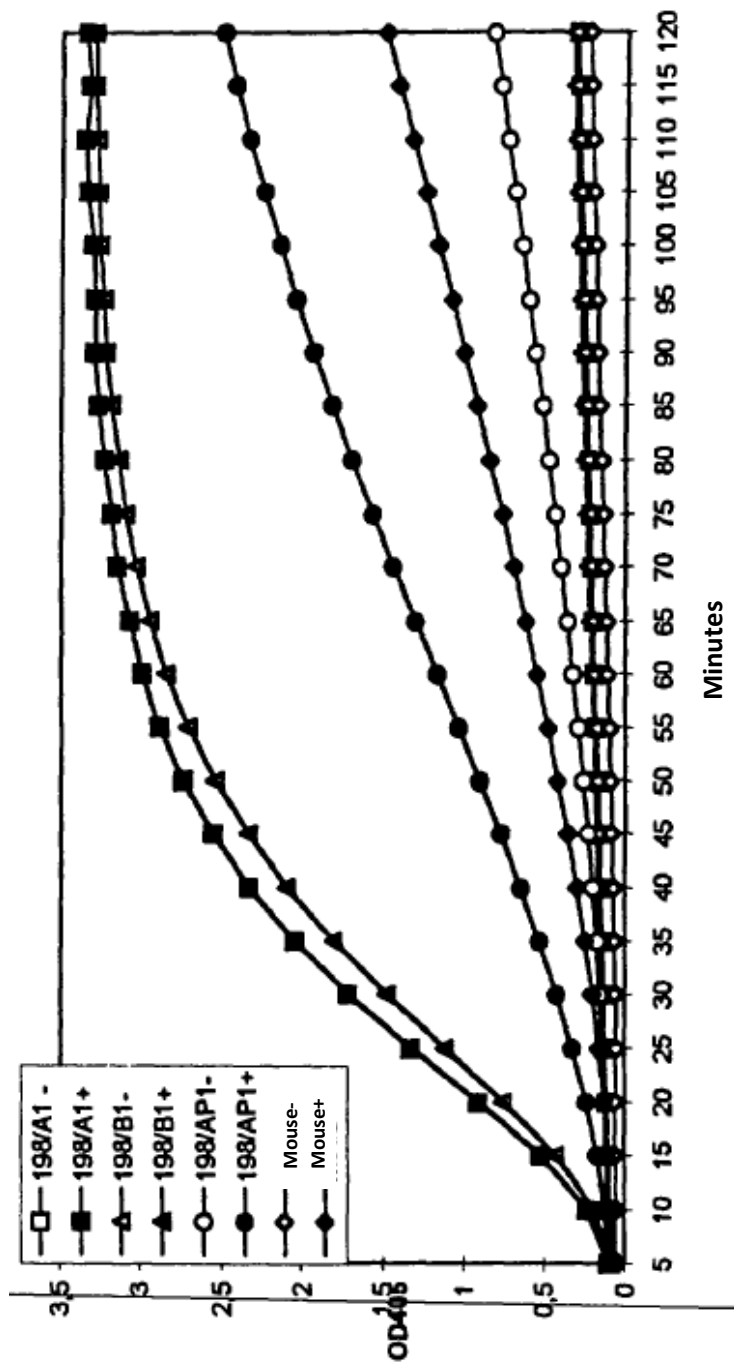


Fig. 11

## Primer for the cloning of mouse $V_H$ genes

*Mouse  $V_H$  reverser primer (containing the SFI1 page):*

VH1BACK-SfiI  
5' C ATG CCA TGA CTC GCG GCC CAG CCG GCC ATG GCC SAG GT'S MAR CTG CAG SAG TCW GG 3'  
VH1BACKSfi  
5' GTC CTC GCA ACT GCG GCC CAG CCG GCC ATG GCC GAG GTG CAG CTT CAG GAG TCA GG 3'  
VH2BACKSfi  
5' GTC CTC GCA ACT GCG GCC CAG CCG GCC ATG GCC GAT GTG CAG CTT CAG GAG TCR GG 3'  
VH3BACKSfi  
5' GTC CTC GCA ACT GCG GCC CAG CCG GCC ATG GCC CAG GTG CAG CTG AAG SAG TCA GG 3'  
VH4/6BACKSfi  
5' GTC CTC GCA ACT GCG GCC CAG CCG GCC ATG GCC GAG GTY CAG CTG CAR TCT GG 3'  
VH5/9BACKSfi  
5' GTC CTC GCA ACT GCG GCC CAG CCG GCC ATG GCC GAG GTY CAR CTG CAG YCT GG 3'  
VH7BACKSfi  
5' GTC CTC GCA ACT GCG GCC CAG CCG GCC ATG GCC GAR GTG AAG CTG GTG GAR TCT GG 3'  
VH8BACKSfi  
5' GTC CTC GCA ACT GCG GCC CAG CCG GCC ATG GCC GAG GTT CAG CTT CAG CAG TCT GG 3'  
VH10BACKSfi  
5' GTC CTC GCA ACT GCG GCC CAG CCG GCC ATG GCC GAA GTG CAG CTG KTG GAG WCT GG 3'  
VH11BACKSfi  
5' GTC CTC GCA ACT GCG GCC CAG CCG GCC ATG GCC CAG ATC CAG TTG CTG CAG TCT GG 3'

**Mouse JH forward primer (containing 1/2 linker sequence and AscI**

VH1FORLiAsc	5' ACC GCC AGA GGC GCG CCC ACC TGA ACC GCC TCC ACC TGA GGA GAC GGT GAC CGT GGT CCC TTG GCC CC 3'
JH1FORLiAsc	5' ACC GCC AGA GGC GCG CCC ACC TGA ACC GCC TCC ACC TGA GGA GAC GGT GAC CGT GGT CCC 3'
JH2FORLiAsc	5' ACC GCC AGA GGC GCG CCC ACC TGA ACC GCC TCC ACC TGA GGA GAC TGT GAG AGT GGT GCC 3'
JH3FORLiAsc	5' ACC GCC AGA GGC GCG CCC ACC TGA ACC GCC TCC ACC TGC AGA GAC AGT GAC CAG AGT CCC 3'
JH4FORLiAsc	5' ACC GCC AGA GGC GCG CCC ACC TGA ACC GCC TCC ACC TGA GGA GAC GGT GAC TGA GGT TCC 3'

IUPAC-Code: M=A/C, W=A/T, R=A/G, Y=C/T, S=C/G, K=G/T, H=A/C/T, D=A/G/T, V=A/C/G, B=T/C/G.

Fig. 12

A1576/99-1

ORIGINAL TEXT

Primer for the cloning of mouse  $V_K$  genes

Mouse  $V_K$  reverse primer (containing the *Ascl* page and  $1/2$  linker sequence)

VK2BACK-LIAscI	5'	GGT	TCA	GAT	GGG	CGC	GCC	TCT	GGC	GGT	GGC	GGA	TCG	GAC	ATT	GAG	CTC	ACC	CAG	TCT	CCA	3'
VK1BACKLI Asc	5'	GGT	TCA	GAT	GGG	CGC	GCC	TCT	GGC	GGT	GGC	GGA	TCG	GAC	ATT	GTG	ATG	WCA	CAG	TCT	CC	3'
VK2BACKLI Asc	5'	GGT	TCA	GAT	GGG	CGC	GCC	TCT	GGC	GGT	GGC	GGA	TCG	GAT	GTT	KTG	ATG	ACC	CAA	ACT	CC	3'
VK3BACKLI Asc	5'	GGT	TCA	GAT	GGG	CGC	GCC	TCT	GGC	GGT	GGC	GGA	TCG	GAT	ATT	GTG	ATR	ACB	CAG	GCW	GC	3'
VK4BACKLI Asc	5'	GGT	TCA	GAT	GGG	CGC	GCC	TCT	GGC	GGT	GGC	GGA	TCG	GAC	ATT	GTG	CTG	ACM	CAR	TCT	CC	3'
VK5BACKLI Asc	5'	GGT	TCA	GAT	GGG	CGC	GCC	TCT	GGC	GGT	GGC	GGA	TCG	GAC	ATT	GTK	CTC	ACC	CAG	TCT	CC	3'
VK6BACKLI Asc	5'	GGT	TCA	GAT	GGG	CGC	GCC	TCT	GGC	GGT	GGC	GGA	TCG	GAT	ATT	VMG	ATG	ACM	CAG	WCT	CC	3'
VK7BACKLI Asc	5'	GGT	TCA	GAT	GGG	CGC	GCC	TCT	GGC	GGT	GGC	GGA	TCG	GAT	ATT	GTT	CTC	ACC	CAG	TCT	CC	3'
VK8BACKLI Asc	5'	GGT	TCA	GAT	GGG	CGC	GCC	TCT	GGC	GGT	GGC	GGA	TCG	TCA	TTA	TTG	CAG	GTG	CTT	GTG	GG	3'

Mouse  $J_K$  forward primer (containing the *NotI* page

JK1NOT10	5'	GAG	TCA	TTC	TGC	GGC	CGC	CCG	TTT	GAT	TTC	CAG	CTT	GGT	GCC	3'
JK2NOT10	5'	GAG	TCA	TTC	TGC	GGC	CGC	CCG	TTT	TAT	TTC	CAG	CTT	GGT	CCC	3'
JK3NOT10	5'	GAG	TCA	TTC	TGC	GGC	CGC	CCG	TTT	TAT	TTC	CAG	TCT	GGT	CCC	3'
JK4NOT10	5'	GAG	TCA	TTC	TGC	GGC	CGC	CCG	TTT	TAT	TTC	CAA	CTT	TGT	CCC	3'
JK5NOT10	5'	GAG	TCA	TTC	TGC	GGC	CGC	CCG	TTT	CAG	CTC	CAG	CTT	GGT	CCC	3'

IUPAC-Code: K=G/T, M=A/C, W=A/T, R=A/G, Y=C/T, S=C/G, H=A/C/T, D=A/G/T, V=A/C/G, B=T/C/G.

Fig. 13

025791

A1576/99-1

ORIGINAL TEXT

VH

+1 E V K L V E S G P E L K K P G  
1 GAG GTG AAG CTG GTG GAG TCT GGA CCT GAG CTG AAG AAG CCT GGA

+1 E T V K I S C K A S G Y I F T  
46 GAG ACA GTC AAG ATC TCC TGC AAG GCT TCT GGG TAT ATC TTC ACA

+1 N Y G M N W V K Q A P G K G L  
91 AAC TAT GGA ATG AAC TGG GTG AAG CAG GCT CCA GGA AAG GGT TTA

+1 K W M G W I N T Y T G E P T Y  
136 AAG TGG ATG GGC TGG ATA AAC ACC TAC ACT GGA GAG CCA ACA TAT

+1 A D D F K G R F A F S L E T S  
181 GCT GAT GAC TTC AAG GGA CGG TTT GCC TTC TCT TTG GAA ACC TCT

+1 A S T A Y L Q I N N L K N E D  
226 GCC AGC ACT GCC TAT TTG CAG ATC AAC AAC CTC AAA AAT GAG GAC

+1 T A T Y F C A L Y G N S P K G  
271 ACG GCT ACA TAT TTC TGT GCA TTA **TAT GGT AAC TCC CCT AAG GGG**

+1 **F A Y W G Q G T L V T V S A G** *linker*  
316 **TTT GCT TAC** TGG GGC CAA GGG ACT CTG GTC ACT GTC TCT GCA **GGT**

VL

+1 G G G S G G R A S G G G G S D  
361 GGA GGC GGT TCA GGT GGG CGC GCC TCT GGC GGT GGC GGA TCG GAT

+1 I Q M T Q S P K F L L V S A G  
406 ATT CAG ATG ACA CAG TCT CCC AAA TTC CTG CTT GTA TCA GCA GGA

+1 D R V T I T C K A S Q S V S N  
451 GAC AGG GTT ACC ATA ACC TGC AAG GCC AGT CAG AGT GTG AGT AAT

+1 D V A W Y Q Q K P G Q S P K L  
496 GAT GTA GCT TGG TAC CAA CAG AAG CCG GGG CAG TCT CCT AAA CTA

+1 L M Y Y A S N R Y T G V P D R  
541 CTG ATG TAC TAT GCA TCC AAT CGC TAC ACT GGA GTC CCT GAT CGC

+1 F T G S G Y G T D F T F T I S  
586 TTC ACT GGC AGT GGA TAT GGG ACG GAT TTC ACT TTC ACC ATC AGC

---

+1 T V Q A E D L A V Y F C **Q Q D**  
631 ACT GTG CAG GCT GAA GAC CTG GCA GTT TAT TTC TGT **CAG CAG GAT**

+1 **Y G S P P T F G G G T K L E I**  
676 **TAT GGC TCT CCT CCC ACG** TTC GGA GGG GGC ACC AAG CTG GAA ATT

+1 K R  
721 AAA CGG

Fig. 14

025791

A1576/99-1

ORIGINAL TEXT

VH

+1 E V Q L V E S G G G L V K P G  
1 GAA GTG CAG CTG GTG GAG TCT GGG GGA GGC CTA GTG AAG CCT GGA

+1 G S L K L S C A A S G F T F S  
46 GGG TCC CTG AAA CTC TCC TGT GCA GCC TCT GGA TTC ACT TTC AGT

+1 T Y T M S W V R Q T P E K R L  
91 ACC TAT ACC ATG TCT TGG GTT CGC CAG ACT CCG GAG AAG AGG CTG

+1 E W V A T I S S G G S Y T Y Y  
136 GAG TGG GTC GCA ACC ATT AGT AGT GGT GGT AGT TAC ACC TAC TAT

+1 P D S V R G R F T I S R D N A  
181 CCA GAC AGT GTG AGG GGC CGA TTC ACC ATC TCC AGA GAC AAT GCC

+1 K N T L Y L Q M S S L K S E D  
226 AAG AAC ACC CTG TAC CTG CAA ATG AGC AGT CTG AAG TCT GAG GAC

+1 T A M Y Y C T R D G G H G Y G  
271 ACA GCC ATG TAT TAC TGT ACA AGA **GAT GGG GGA CAC GGG TAC GGT**

+1 S S F D Y W G Q G T T L T V S  
316 **AGT AGC TTT GAC TAC** TGG GGC CAA GGC ACC ACT CTC ACA GTC TCC

*linker*

+1 S G G G G S G G R A S G G G G  
361 TCA GGT GGA GGC GGT TCA GGT GGG CGC GCC TCT GGC GGT GGC GGA

VL

+1 S Q I V L T Q S P L S L P V S  
406 TCG CAA ATT GTG CTC ACC CAG TCT CCA CTC TCC CTG CCT GTC AGT

+1 L G D Q A S I S C R S S Q S I  
451 CTT GGA GAT CAA GCC TCC ATC TCT TGC AGA TCT AGT CAG AGC ATT

+1 V H S N G N T Y L E W Y L Q K  
496 GTA CAT AGT AAT GGA AAC ACC TAT TTA GAA TGG TAC CTG CAG AAA

+1 P G Q S P K L L I Y K V S N R  
541 CCA GGC CAG TCT CCA AAG CTC CTG ATC TAC AAA GTT TCC AAC CGA

+1 F S G V P D K F S G S G S G T  
586 TTT TCT GGG GTC CCA GAC AAA TTC AGT GGC AGT GGA TCA GGG ACA

---

+1 D E T L K I S R V E A E D L G  
631 GAT TTC ACA CTC AAG ATC AGC AGA GTG GAG GCT GAG GAT CTG GGA

+1 V Y Y C F Q G S H V P W T F G  
676 GTT TAT TAC TGC **TTT CAA GGT TCA CAT GTT CCG TGG ACG** TTC GGT

1 G G T K L E I K R  
721 GGA GGC ACC AAG CTG GAA ATC AAA CGG

Fig. 15



025791

A1576/99-1

ORIGINAL TEXT

VH

+1 E V Q L Q E S G G G L V K P G  
1 GAG GTG CAG CTT CAG GAG TCA GGG GGA GGC TTA GTG AAG CCT GGA

+1 G S L K L S C A A S G F T F S  
46 GGG TCC CTG AAA CTC TCC TGT GCA GCC TCT GGA TTC ACT TTC AGT

+1 S Y T M S W V R Q T P E K R L  
91 AGC TAT ACC ATG TCT TGG GTT CGC CAG ACT CCG GAG AAG AGG CTG

+1 E W V A T I S S G G S S T Y Y  
136 GAG TGG GTC GCA ACC ATT AGT AGT GGT GGT AGT TCC ACC TAC TAT

+1 P D S V K G R F T I S R D N A  
181 CCA GAC AGT GTG AAG GGC CGA TTC ACC ATC TCC AGA GAC AAT GCC

+1 K N T L Y L Q M S S L R S E D  
226 AAG AAC ACC CTG TAC CTG CAA ATG AGC AGT CTG AGG TCT GAG GAC

+1 T A M Y Y C T R E G G G F T V  
271 ACA GCC ATG TAT TAC TGT ACA AGA GAG GGG GGT GGT TTC ACC GTC

+1 N W Y F D V W G A G T L V T V  
316 AAC TGG TAC TTC GAT GTC TGG GGC GCA GGG ACT CTG GTC ACT GTC

*linker*

+1 S A G G G S G G R A S G G G  
361 TCT GCA GGT GGA GGC GGT TCA GGT GGG CGC GCC TCT GGC GGT GGC

VL

+1 G S E N V L T Q S P A S L A V  
406 GGA TCG GAA AAT GTG CTC ACC CAG TCT CCA GCT TCT TTG GCT GTG

+1 S L G Q R A T I S C R A S E S  
451 TCT CTA GGG CAG AGG GCC ACC ATA TCC TGC AGA GCC AGT GAA AGT

+1 V D S Y G Y N F M H W Y Q Q I  
496 GTT GAT AGT TAT GGC TAT AAT TTT ATG CAC TGG TAT CAG CAG ATA

+1 P G Q P P K L L I Y R A S N L  
541 CCA GGA CAG CCA CCC AAA CTC CTC ATC TAT CGT GCA TCC AAC CTA

+1 E S G I P A R F S G S G S R T  
586 GAG TCT GGG ATC CCT GCC AGG TTC AGT GGC AGT GGG TCT AGG ACA

---

+1 D F T L T I N P V E A D D V A  
631 GAC TTC ACC CTC ACC ATT AAT CCT GTG GAG GCT GAT GAT GTT GCA

+1 T Y Y C Q Q S N E D P L T F G  
676 ACC TAT TAC TGT CAG CAA AGT AAT GAG GAT CCG CTC ACG TTC GGT

+1 T G T R L E I K R  
721 ACT GGG ACC AGA CTG GAA ATA AAA CGG

Fig. 16

025791

A1576/99-1

ORIGINAL TEXT

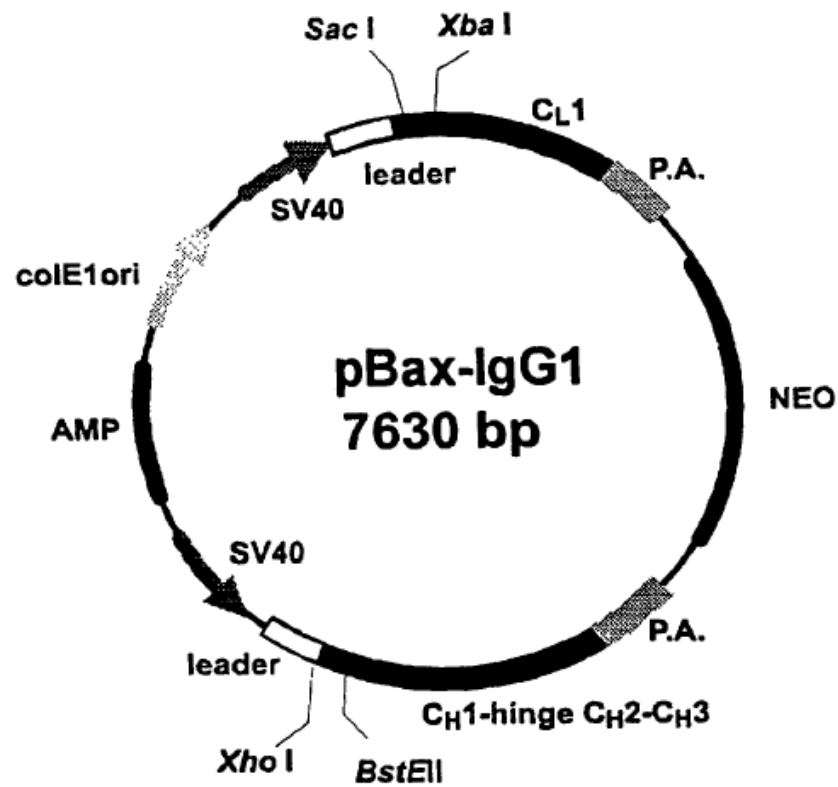


Fig. 17

**IN THE UNITED STATES DISTRICT COURT  
FOR THE DISTRICT OF DELAWARE**

BAXALTA INCORPORATED, a Delaware  
corporation; and BAXALTA GMBH, a Swiss  
company,

Plaintiffs,

v.

GENENTECH, INC., a Delaware corporation; and  
CHUGAI PHARMACEUTICAL CO., LTD., a  
Japanese company,

Defendants.

C.A. No. 17-509-TBD

**BAXALTA'S OPENING *MARKMAN* BRIEF**

activity of Factor IXa”		
“increases the procoagulant activity of Factor IXa”	Increases the rate of clot formation promoted by Factor IXa	Enhances the ability of Factor IXa to activate Factor X to Factor Xa by any amount as determined by any assay used to measure Factor VIII-like activity

The parties agree that “increases” should be given its plain and ordinary meaning. D.I. 131, Amended Joint Chart at 6. They dispute whether “the procoagulant activity of Factor IXa” (i) explicitly incorporates only one of the activation steps of the coagulation cascade,<sup>13</sup> and (ii) requires a particular method to measure that activation step. As their 26-word construction demonstrates, Defendants propose to fundamentally rewrite the claim—a practice that the Federal Circuit has repeatedly rejected. *See Resonate Inc. v. Alteon Websystems, Inc.*, 338 F.3d 1360, 1365 (Fed. Cir. 2003) (“Courts may not rewrite claim language based on what has been omitted from a claim, and the district court's attempt to do so here was legal error.”).<sup>14</sup>

### 1. The Intrinsic Record Supports Baxalta’s Construction

The specification supports Baxalta’s construction that “the procoagulant activity of Factor IXa” is “the rate of clot formation promoted by Factor IXa.” The specification teaches that the inventors tested for procoagulant activity by analyzing the Disclosed Antibodies in a one-stage

<sup>13</sup> The human body uses a complex mechanism called the coagulation cascade to promote the clotting of blood in response to a vascular injury. D.I. 47 ¶ 38. The coagulation cascade includes a series of reactions involving numerous factors, whereby the activation of one factor may lead to the activation of the next factor in the cascade. *See* ‘590 Patent at 1:7-11. During the coagulation cascade, *one step* involves the activation of FX to FXa by the binding of FVIIIa to FIXa to form a tenase-complex. *Id.* at 1:61-65. FXa then cleaves FII (prothrombin) converting it to FIIa (thrombin), and thrombin then cleaves FI (fibrinogen) converting it to FIa (fibrin), which forms a clot. D.I. 47 ¶ 39, Fig. 1.

<sup>14</sup> *Id.* (citing *K-2 Corp. v. Salomon S.A.*, 191 F.3d 1356, 1364 (Fed. Cir. 1999) (“Courts do not rewrite claims; instead, we give effect to the terms chosen by the patentee.”); *Autogiro Co. of Am. v. United States*, 384 F.2d 391, 396 (Ct. Cl. 1967) (“Courts can neither broaden nor narrow the claims to give the patentee something different than what he has set forth.”)).

clotting assay.<sup>15</sup> ‘590 Patent at 18:28-33 (“FIX/FIXa specific monoclonal antibodies selected from the 198th fusion experiment were purified from the respective hybridoma supernatant and quantified as described in Example 3. These antibodies were analyzed in a *modified one-stage clotting assay* (as described in Example 6) and some showed *procoagulant activity*.”); *Id.* at 29:47-51 (“To assay for the *procoagulant activity* of peptide A1/3 in FVIII inhibitor plasma the following experiment was carried out. We performed a standard aPTT based *one stage clotting assay*, but instead of FVIII deficient plasma we employed FVIII inhibitor plasma”).

Moreover, the specification repeatedly discloses that a reduction in clotting time demonstrates an increase in procoagulant activity. *Id.* at 17:36-39 (“There is a clear dose-dependent *reduction of the clotting time* . . . . These results imply that antibody 193/AD3 is *procoagulant* in the presence of FIXa.”); *Id.* at 29:37-40 (“In the presence of FIXa, peptide B1/7 becomes *procoagulant* as indicated by the *reduced clotting time*.”); *Id.* at 23:66-24:2 (“Peptides A1/1, A1/2, A1/4 and A1/5 did not give any reduction in the *clotting time* indicating that they lack *procoagulant activity*.”).

The specification thus teaches that procoagulant activity is assessed by measuring the formation of a clot and that an increase in the procoagulant activity of FIXa is shown by a reduction in clotting time, *i.e.*, by promoting blood coagulation. A reduction in the time it takes to form a clot means that the rate of clotting has increased. Accordingly, the terms “the procoagulant activity of Factor IXa” and “increases the procoagulant activity of Factor IXa” refer to a rate of clot formation promoted by FIXa.

---

<sup>15</sup> A clotting assay measures the final outcome of the coagulation cascade, *i.e.*, the formation of a clot. D.I. 118 ¶ 43.

**2. Defendants Ignore the Intrinsic Record in an Attempt to Import Requirements into the Claims**

Defendants' proposed construction seeks to restrict "procoagulant activity of Factor IXa" to "the ability of Factor IXa to activate Factor X to Factor Xa by any amount" and further requires that such activity be determined by "any assay" for measuring "Factor VIII-like activity." These constructs, however, conflate procoagulant activity with FVIII-like activity and thus rewrite the claims and flout the intrinsic evidence.

At no point do the claims or the specification describe "the procoagulant activity of Factor IXa" as "the ability of Factor IXa to activate Factor X to Factor Xa by any amount." While the specification describes the activation of FX to FXa as *a* step in the coagulation cascade ('590 Patent at 1:61-65), a POSITA would have understood that it is one of many steps that ultimately lead to the formation of a clot. D.I. 111 ¶¶ 35-39. Moreover, the specification does not constrain FIXa's role in the coagulation cascade to only activating FX to FXa. FIXa may also participate in other reactions in the coagulation cascade to promote clot formation. Ex. C, Krishnaswamy Dep. Tr. 79:3-14. Thus, solely examining FIXa's role in the enzymatic conversion of FX to FXa, one of the many enzymatic conversions in the coagulation cascade, as Defendants advance, would exclude other mechanisms by which the claimed antibody or antibody fragment thereof may increase the procoagulant activity of FIXa.

The specification also does not require procoagulant activity be determined by "any assay used to measure Factor VIII-like activity," as it makes clear FVIII-like activity and procoagulant activity are distinct. Indeed, the specification provides that methods may be used to show that the antibodies of the present invention "increase the procoagulant activity of factor IXa *or* have factor

VIII-like activity.” ‘590 Patent at 9:17-18.<sup>16</sup>

Moreover, the specification teaches that an antibody may exhibit FVIII-like activity *without* exhibiting procoagulant activity. For example, the disclosed 198/AP1 antibody, which exhibited FVIII-like activity in a chromogenic assay, did *not* exhibit procoagulant activity in an aPTT assay. *Id.* at 18:34-63, Fig. 11; D.I. 128 ¶¶ 51. Since different assays may provide conflicting results, Defendants’ construction requiring “any assay” cannot be correct. *See Geneva Pharms., Inc. v. GlaxoSmithKline PLC*, 349 F.3d 1373 (Fed. Cir. 2003) (a claim construction that renders claims indefinite is incorrect).

**G. Claim Terms: “preparation comprising an antibody or antibody fragment ... and a pharmaceutically acceptable carrier” and “pharmaceutically acceptable carrier”**

Term	Baxalta’s Construction	Defendants’ Construction
“preparation comprising an antibody or antibody fragment ... and a pharmaceutically acceptable carrier”	The claimed preparation must be suitable for pharmaceutical use	The claimed preparation must be therapeutically useful
“pharmaceutically acceptable carrier”	Suitable for pharmaceutical use	A substance suitable for administration to a patient in which a therapeutically useful agent is dissolved or suspended

**1. Baxalta’s Constructions Should Be Adopted**

The plain language of the term “*pharmaceutically* acceptable carrier” supports Baxalta’s proposed constructions because the carrier’s acceptability is contingent on its pharmaceutical

<sup>16</sup> The foreign priority application of the ‘590 Patent also distinguishes between procoagulant activity and FVIII-like activity. *Compare* D.I. 129-3, Austrian Patent Application No. A1576/99 at cl. 1 *with* cl. 2; D.I. 128 ¶¶ 54-56. Claim 1 is drawn to “procoagulant activity of FIXa” while claim 2 is drawn to “FVIIIa (FVIIIa)-like cofactor activity.” *Id.* The inventors identified different tests for the different activities. They used an aPTT test to measure the “procoagulant activity of the antibody 193/AD3” and a chromogenic assay to measure “FVIII-like activity of the antibodies 193/AD3 and 196/AF2.” D.I. 129-3 at 27, 28, 31, Figs. 6A, 9.

**CONFIDENTIAL MATERIAL OMITTED  
FROM THE FOLLOWING PAGES:**  
Appx11236, Appx11434



**IN THE UNITED STATES DISTRICT COURT  
FOR THE DISTRICT OF DELAWARE**

BAXALTA INCORPORATED and  
BAXALTA GMBH,

Plaintiffs,

v.

GENENTECH, INC. and CHUGAI  
PHARMACEUTICAL CO., LTD.,

Defendants.

Civil Action No. 17-509-TBD

**OPINION & ORDER**

On May 4, 2017, Baxalta Inc. and Baxalta GmbH (together, “Baxalta”) brought suit against Genentech, Inc. and Chugai Pharmaceutical Co., Ltd., alleging infringement of U.S. Patent No. 7,033,590 (“the ‘590 patent”) by the manufacture, use, sale, offer to sell, and importation of an antibody used to treat hemophilia A and known as emicizumab or ACE910, marketed under the brand name Hemlibra. On December 14, 2017, Baxalta moved for a preliminary injunction barring further sales or offers to sell Hemlibra in the United States, with exceptions for certain patients. In addition to two rounds of briefing, the Court held an evidentiary hearing on Baxalta’s motion on June 13 and 14, 2018, and heard oral argument on July 2, 2018. Baxalta having failed to meet its burden for showing the propriety of preliminary injunctive relief, the motion for a preliminary injunction is DENIED. This opinion constitutes the Court’s findings of fact and conclusions of law pursuant to Federal Rule of Civil Procedure 52(a).

Case 1:17-cv-00509-TBD Document 275 Filed 08/10/18 Page 1 of 1 PageID #: 22061

**IN THE UNITED STATES DISTRICT COURT  
FOR THE DISTRICT OF DELAWARE**

BAXALTA INCORPORATED and  
BAXALTA GMBH,

Plaintiffs,

v.

GENENTECH, INC. and CHUGAI  
PHARMACEUTICAL CO., LTD.,

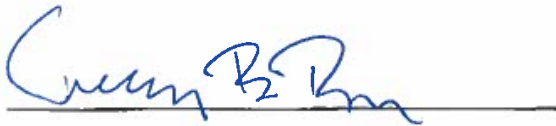
Defendants.

Civil Action No. 17-509-TBD

**ORDER**

On consideration of the parties' joint letter, dated July 18, 2018, ECF No. 245, and Genentech's letter, dated July 23, 2018, ECF No. 247, the documents listed in Exhibit A to the parties' joint letter, ECF No. 245-1, are ADMITTED and are part of the preliminary injunction record. The parties' objections to inclusion of these documents are OVERRULED.

**IT IS SO ORDERED** this 10th day of August, 2018.

A handwritten signature in blue ink, appearing to read "Timothy B. Dyk", is written over a horizontal line.

Honorable Timothy B. Dyk  
United States Circuit Judge, sitting by designation

**IN THE UNITED STATES DISTRICT COURT  
FOR THE DISTRICT OF DELAWARE**

BAXALTA INCORPORATED, a Delaware  
corporation; and BAXALTA GMBH, a Swiss  
company,

Plaintiffs,

v.

GENENTECH, INC., a Delaware corporation; and  
CHUGAI PHARMACEUTICAL CO., LTD., a  
Japanese company,

Defendants.

C.A. No. 1:17-cv-00509-TBD

JURY TRIAL DEMANDED

**FIRST AMENDED STIPULATION AND ORDER DISMISSING  
DEFENDANT CHUGAI PHARMACEUTICAL CO., LTD.**

Plaintiffs Baxalta Incorporated and Baxalta GmbH (collectively, “Baxalta”) and Defendant Chugai Pharmaceutical Co., Ltd. (“Chugai”) submit the following Stipulation dismissing Defendant Chugai (the “Stipulation”) and in support thereof state:

1. On June 26, 2018, Baxalta and Chugai submitted a “Stipulation and [Proposed] Order as to Defendant Chugai Pharmaceutical Co., Ltd.,” in which, *inter alia*, Chugai agreed to provide one or more witnesses for a Rule 30(b)(6) deposition, and in which Baxalta agreed to dismiss Chugai from this Action within five business days following completion of the 30(b)(6) deposition.

2. On July 2, 2018, the Court ordered that “[w]ithin five days of completion of the deposition, Baxalta shall file a proposed order dismissing Chugai in accordance with the stipulation.”

3. On September 5 and 6, 2018, in Osaka, Japan, Baxalta deposed a Chugai witness on agreed-upon 30(b)(6) topics. The deposition was completed on September 6, 2018.

**IN THE UNITED STATES DISTRICT COURT  
FOR THE DISTRICT OF DELAWARE**

BAXALTA INCORPORATED and  
BAXALTA GMBH,

Plaintiffs,

v.

GENENTECH, INC. and CHUGAI  
PHARMACEUTICAL CO., LTD.,

Defendants.

Civil Action No. 17-509-TBD

**OPINION & ORDER**

On May 4, 2017, Baxalta Inc. and Baxalta GmbH (together, “Baxalta”) filed suit against Genentech, Inc. and Chugai Pharmaceutical Co., Ltd. alleging infringement of claims 1, 4, 17, and 19 of U.S. Patent No. 7,033,590 patent (“the ‘590 patent”).<sup>1</sup> Chugai was voluntarily dismissed from this lawsuit pursuant to a stipulation of the parties on September 19, 2018. Order Dismissing Chugai, ECF No. 293.

The alleged infringement is the manufacture, use, sale, offer to sell, and importation of an antibody used to treat hemophilia A and known as emicizumab, or ACE910, and marketed under the brand name Hemlibra (hereinafter, “Hemlibra”). Now before this court is the claim construction of six terms of the ‘590 patent: *antibody*, *antibody fragment*, *bispecific antibody*, *isolated*, *binds Factor IX or Factor IXa and increases*, and *increases the procoagulant activity of Factor IXa*.

---

<sup>1</sup> Baxalta also initially asserted infringement of claim 15 of the ‘590 patent, but dropped this claim during the Markman hearing. See First Am. Compl. 9, ECF No. 239; Markman Tr. 235:11–13, ECF No. 320.

This court held a Markman hearing on October 16, 2018, and received expert testimony and argument regarding the construction of the six terms. At an earlier preliminary injunction hearing on June 13 and 14, 2018, the court also received testimony and argument on construction of the terms *antibody* and *antibody fragment*.

In terms of the factual record, the court will consider oral testimony given by experts at the Markman hearing, testimony offered at the preliminary injunction hearing, and the deposition testimony and reports and declarations of any of those experts, but the court declines to consider the declarations of experts who have not been subject to cross-examination at either hearing.<sup>2</sup> Whether or not such declarations are considered makes no difference to the constructions adopted by the court here.

## BACKGROUND

### I. PROCEDURAL HISTORY

On May 4, 2017, Baxalta filed its complaint alleging infringement of the '590 patent. Compl. ¶¶ 37–51, ECF No. 1. On June 30, Genentech answered, denying Baxalta's allegations and counterclaiming for declaratory judgment of noninfringement and invalidity. Answer & Countercl. ¶¶ 37–51, 120–49, ECF No. 9.

On December 14, 2017, Baxalta moved for a preliminary injunction. Mot. Prelim. Inj. 2, ECF No. 41; Prop. Prelim. Inj. Order 1, ECF No. 42-1. After an evidentiary hearing on August

---

<sup>2</sup> Baxalta initially presented Dr. Anthony A. Kossiakoff's declaration in support of its claim construction positions. *See* Kossiakoff Reb. Decl., ECF No. 126. I postponed the date of this Markman hearing three times and made clear to the parties that the hearing was their opportunity to present expert witness testimony in support of their claim construction positions. *See* May 14, 2018 Minute Entry; Sept. 12, 2018 Oral Order; Sept. 25, 2018 Oral Order. Nonetheless, Baxalta failed to offer testimony from Dr. Kossiakoff at the Markman hearing, and Genentech accordingly objected to reliance on Dr. Kossiakoff's declaration. Markman Tr. 46:1–22, 53:12–54:11. I sustained this objection and do not consider Dr. Kossiakoff's declaration for the purposes of claim construction. Markman Tr. 54:12–55:3. As noted, the Kossiakoff Declaration, even if considered, would make no difference in the outcome.

7, 2018, this court denied Baxalta's preliminary injunction motion. Prelim. Inj. Order at 29, ECF No. 262. In that connection, the court declined to construe *antibody* and *antibody fragment*, concluding that the parties had presented "substantial arguments" on both sides. *Id.* at 13. Baxalta did not appeal the denial of the preliminary injunction. Discovery has been ongoing. Fact discovery is set to close on December 14, 2018, and expert discovery is set to close on April 19, 2019. Stip. & Order Amend. Sched. 1, ECF No. 325.

At the Markman hearing, the parties presented expert testimony and argued construction of six terms: *antibody*, *antibody fragment*, *bispecific antibody*, *isolated*, *binds Factor IX or Factor IXa and increases*, and *increases the procoagulant activity of Factor IXa*. All these terms appear in claims 1 and 4 of the '590 patent, which recite

1. An isolated antibody or antibody fragment thereof that binds Factor IX or Factor IXa and increases the procoagulant activity of Factor IXa.

\* \* \*

4. The antibody or antibody fragment according to claim 1, wherein said antibody or antibody fragment is selected from the group consisting of a monoclonal antibody, a chimeric antibody, a humanized antibody, a single chain antibody, a bispecific antibody, a diabody, and di-, oligo- or multimers thereof.

'590 patent, col. 101, ll. 43–45, 51–56 (underlining added).<sup>3</sup>

---

<sup>3</sup> Claims 17 and 19 of the '590 patent, also at issue here, are as follows:

17. A method of obtaining an antibody that interacts with Factor IX or Factor IXa and increases the procoagulant activity of Factor IXa, comprising the steps of:  
Immunizing an immunocompetent mouse with an antigen selected from the group consisting of FIX, FIXα, FIXαβ or fragments thereof,  
isolating spleen cells of the immunized mouse,  
producing hybridoma cells,  
screening the hybridoma cell supernatants for an increase in the procoagulant activity of Factor IXa, isolating and purifying the antibody from a supernatant from the hybridoma cells which exhibit an increase in the procoagulant activity of Factor IXa.

## II. COAGULATION & HEMOPHILIA A

In general, the term *antibody* is used to describe glycoproteins that are “characterized by their ability to bind both to antigens and to specialized cells or proteins of the immune system.” Strohl Decl. ¶ 22, ECF No. 112; *accord* Almagro Decl. ¶ 33, ECF No. 49. Structurally, antibodies are Y-shaped, with two arms that are connected by disulfide bonds. Almagro Decl. ¶ 34; Strohl Decl. ¶ 22. Each arm of the Y contains two polypeptide chains known as the heavy (“H”) chain and the light (“L”) chain.<sup>4</sup> Almagro Decl. ¶ 34; Strohl Decl. ¶ 22. The portions of the heavy chain and light chain that are responsible for binding an antigen are called variable domains, V<sub>H</sub> and V<sub>L</sub> respectively. Almagro Decl. ¶ 34; Strohl Decl. ¶ 23. The remaining portions of the antibody are made up of constant regions. Almagro Decl. ¶ 34; Strohl Decl. ¶ 23.

Within each variable domain, the antigen binding sequence of the antibody is divided into three regions called complementarity-determining regions (“CDRs”). Almagro Decl. ¶ 35; Strohl Decl. ¶ 25. The three CDRs in each variable region—designated CDR1, CDR2, and CDR3—determine the binding specificity of the antibody. Almagro Decl. ¶ 35; Strohl Decl. ¶ 25. The CDR3 region of the heavy chain variable domain is “primarily responsible for antigen binding specificity.” Strohl Decl. ¶ 25; *accord* Almagro Decl. ¶ 38.

While the parties agree as to these characteristics of an antibody, they disagree in at least one critical respect. Genentech contends that, as used in the patent, the term *antibody* standing

---

\* \* \*

19. The antibody or antibody fragment according to claim 4, wherein the antibody is a humanized antibody.

<sup>3</sup> 590 patent, col. 103, l. 3–col. 104, l. 6.

<sup>4</sup> Though the basic unit of an antibody is a Y-shaped structure with two L chains and two H chains, certain types of antibodies are more complex and can have up to ten light and ten heavy chains. Markman Tr. 67:4–21.

alone has two heavy chains that are identical and the two light chains that are identical. Genentech Op. Br. 6–8, ECF No. 160; Strohl Decl. ¶ 50. Baxalta, in contrast, argues that the heavy chains are not necessarily identical to one another and the light chains are also not necessarily identical to one another. Baxalta Op. Br. 4–5, ECF No. 158. The resolution of this difference appears to be determinative of infringement.

Hemophilia A and the process of blood coagulation are described at length in the preliminary injunction order. *See* Prelim. Inj. Order 3–4. Relevant here is one particular step of the clotting cascade involving Factor VIIIa and Factor IXa. *See* Aledort Decl. ¶ 13, ECF No. 46. In healthy individuals, Factor VIIIa and Factor IXa form a complex, which allows Factor IXa to activate Factor X. *See id.*; Sheehan Decl. ¶ 36, ECF No. 111. In patients afflicted with hemophilia A, Factor VIII is reduced, defective, or absent. *See* Aledort Decl. ¶ 14; Sheehan Decl. ¶ 42. This hinders the coagulation cascade by limiting the body’s ability to activate Factor X. Aledort Decl. ¶ 14; Sheehan Decl. ¶ 42.

Genentech’s drug, Hemlibra, is directed to this step of the coagulation cascade and functions by replacing Factor VIIIa. *See* Krishnaswamy Decl. ¶ 61, ECF No. 47. Hemlibra does not have both identical light and identical heavy chains. *See* Strohl Decl. ¶¶ 38, 53; *see also* Krishnaswamy Decl. ¶¶ 55, 60. It is a bispecific antibody. The parties agree that in this patent a *bispecific antibody* has non-identical light chains, or non-identical heavy chains, or both.<sup>5</sup> Markman Tr. 38:5–21, ECF No. 320. One arm of the Hemlibra antibody binds to Factor IX (or IXa) and the other binds to Factor X. *See* Krishnaswamy Decl. ¶¶ 55, 60; Strohl Decl. ¶ 53. By

---

<sup>5</sup> During the Markman hearing, Dr. Almagro testified that “there were some examples [of bispecific antibodies] before 1999 with identical light chains and identical heavy chains.” Markman Tr. 109:16–18, ECF No. 320. Nonetheless, the parties have agreed that the bispecific antibodies in the ’590 patent are outside the scope of the column 5 definition of *antibody*. *Id.* 108:11–15, 109:19–110:8 (Almagro testimony).



doing so, Hemlibra allows Factor IX to activate Factor X. *See* Krishnaswamy Decl. ¶ 61, Strohl Decl. ¶¶ 178–79.

## ANALYSIS

### 1. *antibody*

**Baxalta’s proposed construction:** A molecule having a specific amino acid sequence comprising two heavy chains (H chains) and two light chains (L chains).

**Genentech’s proposed construction:** An immunoglobulin molecule, having a specific amino acid sequence that only binds to the antigen that induced its synthesis or very similar antigens, consisting of two identical heavy chains (H chains) and two identical light chains (L chains).

**Court’s construction:** An immunoglobulin molecule, having a specific amino acid sequence that only binds to the antigen that induced its synthesis or very similar antigens, consisting of two identical heavy chains (H chains) and two identical light chains (L chains).

Construing the claims requires resolution of the parties’ primary dispute that an *antibody* in the claims is required to have two identical heavy chains and two identical light chains.<sup>6</sup> The parties agree that the requirement that an antibody have two identical heavy chains and two identical light chains would exclude Hemlibra from the scope of the term *antibody*. Prelim. Inj. Tr. 9:15–24, ECF No. 214–15; Markman Tr. 109:23–110:8. Hemlibra is not an *antibody* under Genentech’s definition.

#### a. *The Meaning of the Term Antibody in the Patent as Originally Drafted*

It is clear from the ’590 patent’s specification that, as originally drafted, the term *antibody* in the claims required identical heavy and identical light chains.

Based on the evidence, I find that the term *antibodies* does not have a single fixed meaning in the art. The word *antibody* can denote different meanings to a person skilled in the

---

<sup>6</sup> A secondary dispute is whether an antibody must bind only to an antigen that induced its synthesis or very similar antigens. While the broader definition of *antibody* would not imply such a requirement, I find that, since the column 5 definition includes this limitation, it is part of the definition of the term *antibody* in the claims. *See* ’590 patent, col. 5, ll. 56–60. It is not clear that this makes any difference with respect to infringement or invalidity.

art depending on the context in which it appears. For example, *antibody* standing alone may connote a different meaning than when it is part of a larger term that defines its structure—e.g., bispecific *antibody*.

The parties agree that the term *antibody* standing alone without other structural terms can have different meanings to those skilled in the art. See Markman Tr. 174:21–175:24. One definition is Baxalta’s definition (hereinafter the “broader” definition), requiring only a molecule with a specific amino acid sequence and comprising two heavy chains and two light chains. The other definition is Genentech’s definition (hereinafter the “narrower” definition), requiring a pair of identical heavy chains and a pair of identical light chains. Baxalta argues that its broader definition should apply because it would have been utilized by persons of ordinary skill in the art. Baxalta Op. Br. 5. But in its opening preliminary injunction brief, Baxalta itself used the narrower definition, stating that “[a]n antibody comprises two identical heavy chains and two identical light chains.” Baxalta Op. Prelim. Inj. Br. 13 n.7, ECF No. 42. Similarly, Dr. Almagro, Baxalta’s expert, described an antibody in his declaration as “a glycoprotein that has a specific ‘Y’ shape” that “has two pairs of identical polypeptide chains, which are linked together by disulfide bonds.” Almagro Decl. ¶ 34. Genentech’s expert agreed that such a definition “represents the plain and ordinary meaning of ‘antibody’ and is consistent with standard textbook definitions of immunoglobulin molecules dating from prior to the 1999 priority date of the ’590 [p]atent.” Strohl Cl. Const. Decl. ¶¶ 41–42, ECF No. 161; accord *id.* ¶ 44.

Various references cited on the face of the ’590 patent use a similar definition. The Roitt reference describes an antibody as “a unit consisting of two identical light polypeptide chains and two identical heavy polypeptide chains.” Ivan Roitt et al., IMMUNOLOGY 72 (5th ed. 1998), Strohl Decl. Ex. F, ECF No. 112-6. And the Harlow and Lane reference says that “[e]ach Y

contains four polypeptides[:] [t]wo identical copies of a polypeptide known as the heavy chain and two identical copies of a polypeptide called the light chain.” Ed Harlow & David Lane, *ANTIBODIES: A LABORATORY MANUAL* 7 (1988), Strohl Decl. Ex. E, ECF No. 112-5. Baxalta does not point to any references cited in the ’590 patent that use a broader definition.

Nonetheless the parties agree that Baxalta’s broader definition was also known to those skilled in the art. *See* Markman Tr. 172:6–176:6. During the Markman hearing, Dr. Strohl, Genentech’s expert testified that the broader definition was a “common language definition.” *Id.* at 175:12–13. Baxalta only points to Dr. Strohl’s testimony as evidence of the understanding of someone skilled in the art.<sup>7</sup>

In the patent specification, the applicant chose the narrower definition. In relevant part, the summary of the invention provides that

Antibodies are immunoglobulin molecules having a specific amino acid sequence which only bind to antigens that induce their synthesis (or its immunogen, respectively) or to antigens (or immunogens) which are very similar to the former. Each immunoglobulin molecule consists of two types of polypeptide chains. Each molecule consists of large, identical heavy chains (H chains) and two light, also identical chains (L chains).

’590 patent, col. 5, ll. 56–63. The Federal Circuit has recognized that use of the verb “is” may “signify that a patentee is serving as its own lexicographer.” *Sinorgchem Co. v. Int’l Trade Comm’n*, 511 F.3d 1132, 1136 (Fed. Cir. 2007) (quoting *Abbott Labs. v. Andrx Pharms., Inc.*, 473 F.3d 1196, 1210 (Fed. Cir. 2007)). The use of the term “are” here is the equivalent of the

---

<sup>7</sup> Even the expert declaration from Dr. Kossiakoff that Baxalta initially offered in support of its construction, but which I have excluded from consideration, does not elaborate on why a person skilled in the art would have understood the term *antibody* to have the broader meaning offered by Baxalta. *See* Kossiakoff Reb. Decl. ¶¶ 21–52. Dr. Kossiakoff’s only statement to this effect is that “a POSITA reviewing the intrinsic record would have understood that the term ‘antibody’ has a plain and ordinary meaning in the art and would not need to be construed,” but that “to the extent a construction is required, it is my opinion that Baxalta’s straightforward construction should be adopted.” *Id.* ¶ 22.

term “is.” Here, the specification unequivocally states what “[a]ntibodies are.” ’590 patent, col. 5, ll. 56–63. This definition is also clearly defining the term *antibodies* covered by the claims of this patent because the definition immediately follows and immediately precedes references to “the inventive antibodies and antibody derivatives.” ’590 patent, col. 5, l. 53; *id.* col. 6, l. 1. The fact that the applicants chose to include the narrower definition in the specification over a broader definition confirms that the applicants intended the narrow definition apply to the term *antibody* standing alone.

Baxalta argues that the specification in other places uses the broader definition. Baxalta Op. Br. 6–7. For example, the specification and claims disclose bispecific antibodies, which do not have identical heavy and light chains. *See id.*; ’590 patent col. 6, ll. 1–5; *id.* col. 7, ll. 32–35; *id.* col. 101, ll. 51–56 (claim 4). Baxalta also points to the inclusion of IgM antibodies and IgA antibodies in claims 3 and 20 as well as throughout the specification. ’590 patent, col. 6, ll. 35–38; *id.* col. 12, ll. 25–26; *id.* col. 14, l. 22–col. 15, l. 4 (Example 4); *id.* col. 30, l. 11–col. 31, l. 10 (Example 13); *id.* col. 101, ll. 49–50 (claim 3); *id.* col. 104, ll. 6–7 (claim 20). IgM and IgA antibodies can have more than two heavy chains and more than two light chains. Markman Tr. 71:11–13, 156:12–159:11. Baxalta thus contends that this limitation of the narrow definition of *antibody* is inappropriate in the context of the ’590 patent. Baxalta Op. Br. 6–7. But all these embodiments were initially listed as falling within “antibodies or antibody derivatives.” *See, e.g.*, ’590 patent, col. 5, l. 51; U.S. Patent App. No. 09/661,992, at 10–11, Strohl Decl. Ex. D, ECF No. 112-4. Thus, as originally drafted, the claims covered antibodies as more broadly defined, but not because they fell within the term *antibody* but because they fell within the term *antibody derivative*.

Under such circumstances, the Federal Circuit has held that the specification's choice of definition governs. *See Sinorgchem*, 511 F.3d at 1136–40 (“Where, as here, multiple embodiments are disclosed, we have previously interpreted claims to exclude embodiments where those embodiments are inconsistent with unambiguous language in the patent's specification or prosecution history.”); *Irdeto Access, Inc. v. Echostar Satellite Corp.*, 383 F.3d 1295, 1300 (Fed. Cir. 2004) (concluding that the scope of the claim terms was controlled by the specification even in the absence of express definitions where applicant admitted to the examiner that the terms had “no accepted meaning in the art” and were “adequately described in the specification”). Indeed, given the specification's clarity, the definition included in column 5 of the '590 patent would govern even if it were contrary to an ordinary meaning of the term. *See Thorner v. Sony Comp. Entmt. Am. LLC*, 669 F.3d 1362, 1365–66 (Fed. Cir. 2012) (“[T]he inventor's written description of the invention, for example, is relevant and controlling insofar as it provides *clear lexicography* . . . .” (alterations and emphasis in original) (quoting *C.R. Bard, Inc. v. U.S. Surgical Corp.*, 388 F.3d 858, 862 (Fed. Cir. 2004))).

Before turning to the prosecution history, it is important to ascertain the meaning of the term *antibody derivative* in the patent as initially drafted. The parties agree that *antibody derivative* is not a term that is commonly used in the art. Markman Tr. 119:21–120:3. But Dr. Almagro, Baxalta's expert, admitted during the preliminary injunction hearing, and again during the Markman hearing, that antibodies that have been altered in some significant way are “sometimes . . . called derivatives.” Prelim. Inj. Tr. 413:4–15; *accord* Markman Tr. 120:6–11 (Dr. Almagro agreeing with previous testimony that “significant variants” of antibodies are “sometimes . . . called derivatives”). Also in support of this understanding, Dr. Almagro, a person of skill in the art, repeatedly described Hemlibra, a bispecific antibody, as being

“derived” from other antibodies.<sup>8</sup> Almagro Decl. ¶ 54 n. 5, ¶ 87 n. 17; Markman Tr. 122:8–24; *see also id.* 120:6–21 (Dr. Almagro agreeing that “[t]alking in plain English” Hemlibra” is “derived from a Factor IX antibody and a Factor X antibody”). This definition of *antibody derivatives*—an antibody that has been altered in some significant way—is consistent with the specification. First, the specification makes clear that the group consisting of *antibodies* and *antibody derivatives* includes bispecific antibodies and other structures that do not have identical light and heavy chains. Since bispecific antibodies are not within the definition of *antibodies*, they must be within the definition of *antibody derivatives*.

This understanding is further supported by the uses of *antibody derivative* in those places in the patent specification where *antibody derivative* is used separately. *Antibody derivative* is used separately in three instances that inform the interpretation of the term<sup>9</sup>: (1) in Example 10, which is entitled “Structure and Procoagulant Activity of Antibody Derivatives Derived from Anti-FIX/FIXa-antibodies; Subcloning Antibody Variable Domains from Hybridoma Cell Lines,” *id.* col. 19, ll. 3–7; (2) in the body of Example 11, where the patent includes in a list of examples of antibody derivatives “scFv, Fab, etc.,” *id.* col. 20, l. 36; and (3) again in Example 13, where the patent refers to “antibody derivatives such as Fab, F(ab)<sub>2</sub>, scFv, etc.,” *id.* col. 30, ll. 16–17. *See also* Markman Tr. 13:19–15:6.

These uses make clear that Fab, F(ab)<sub>2</sub>, and scFv are all antibody derivatives. As Dr. Almagro and Dr. Strohl agree, Fab and F(ab)<sub>2</sub> are the sort of canonical *antibody fragments* (a subset of derivatives) that a person of skill in the art would unquestionably have understood as

<sup>8</sup> In a similar vein, Dr. Strohl testified that “a chimeric antibody would be derived and be a derivative.” Markman Tr. 172:8–9.

<sup>9</sup> The specification also provides that “antibody derivatives may . . . be prepared by means of methods known from the prior art, e.g. by molecular modeling.” ’590 patent, col. 9, ll. 5–7. This use of *antibody derivative*, however, does not assist in understanding the term.

such. Markman Tr. at 117:7–14 (Dr. Almagro), 155:19–23 (Dr. Strohl). Each of these can be derived from an existing *antibody* as defined in the specification. A Fab comprises “the complete light chain[] paired with the full variable and a portion of the constant domain[] of the heavy chain[]” and can be excised from an existing antibody. *See* Strohl Claim Const. Decl. ¶ 31 & Fig. 2, ECF No. 161; *accord* Markman Tr. 151:18–152:24 (Dr. Strohl). A F(ab)<sub>2</sub> comprises “two Fab fragments linked with disulfide bonds” and also “can be generated from an antibody by cleaving off the other portions” to leave the fragment remaining. Markman Tr. 152:25–153:11 (Dr. Strohl); *accord* Strohl Decl. ¶ 32 & Fig. 3. Based on inclusion of Fab and F(ab)<sub>2</sub> it is clear that *antibody fragments* are *antibody derivatives*.

But the specification makes clear that an scFv is not an *antibody fragment* using the definition of *antibody* from the specification. Rather, it is called a single-chain variable fragment and is synthetically created by linking with a stretch of synthetic peptide “a truncated fragment comprising only the [variable heavy] domain” of an antibody with a truncated fragment comprising only the variable light region of an antibody. Strohl Claim Const. Decl. ¶ 33 & Fig. 4; *accord* Almagro Decl. ¶ 35; Markman Tr. 60:2–6, 201:17–22. Because of the reference to scFv as an *antibody derivative*, the term *antibody derivative* was clearly meant to include *antibodies* that have been altered in some significant way.

Thus, I find that the term *antibody derivative* was used in the patent to denote *antibodies* within the column 5 definition that had been altered in some significant way. As initially drafted, there was no inconsistency between the dependent claims and the column 5 definition of *antibody*.

***b. The Prosecution History's Exclusion of Antibody Derivatives Confirms the Specification's Definition of Antibody***

During prosecution the Examiner found various categories of derivatives other than antibody fragments not enabled. The applicants disclaimed *antibody derivatives* including bispecific antibodies, except *antibody fragments*.

Initially, the patent specification and the accompanying claims repeatedly referred to “antibodies and antibody derivatives”; the patent did not refer to “antibody fragments.” See U.S. Patent App. No. 09/661,992, Strohl Decl. Ex. D, ECF No. 112-4. In an office action dated January 2, 2004, the Examiner rejected claims 1–14, 16, 18–19, 23, and 27 of the ’590 patent for lack of enablement. Jan. 2, 2004 Rejection, Strohl Decl. Ex. K, at 4, ECF No. 112-11. The Examiner again rejected these claims for near-identical reasons on September 13, 2004. Sept. 13, 2004 Rejection, Strohl Decl. Ex. M, at 2–3, ECF No. 112-13. To respond to those rejections, the term *antibody derivatives* was deleted by an amendment to the claims, and the term *antibody fragment* was added. Compare U.S. Patent App. No. 09/661,992 with ’590 patent.

It is useful to describe how these amendments came about. Considering the prosecution history as a whole, save for the failure to conform the language of certain dependent claims (discussed below), it is apparent that the applicants and the Examiner continued to view the term *antibody* as having its original meaning from the specification, but that antibody derivatives (except antibody fragments) were now excluded from the scope of the claims. As noted, during prosecution, the claims were initially rejected by the Examiner as not enabled. Jan. 2, 2004 Rejection, at 4. The Examiner found enabled certain *antibody derivatives* “wherein the variable region of said antibody derivative comprises” specific portions of certain amino acid sequences (SEQ ID NOs 82, 84, and 86) disclosed in the patent. *Id.* But the Examiner found not enabled *antibody derivatives* comprising different portions of the same amino acid sequences. *Id.*



Among the *antibody derivatives* the Examiner found not enabled were those comprising “chimeric antibodies, humanized antibodies, single chain antibodies, bispecific antibodies, diabodies and di-, oligo- or multimers thereof in claim 4.” *Id.* Based on this lack of enablement, the claims were rejected.

Dr. Strohl explained during the Markman hearing that the portions of SEQ ID NOs 82, 84, and 86 found enabled by the Examiner comprised excised portions of already-existing antibodies as defined in the specification, that is, antibody fragments. *See* Markman Tr. 200:5–201:22; *id.* at 203:5–205:16. The portions found not enabled were engineered artificial linker sequences—i.e., human-engineered, synthetic peptides. *Id.* 203:5–205:16; *see also* Strohl Decl. ¶ 30 (discussing the composition of scFvs).

The applicants responded to the Examiner’s rejection on July 2, 2004, and argued that the disclosed “antibodies and antibody derivatives” were all enabled because the specification “provides extensive discussion regarding methods of preparing claimed antibodies” and “provides the complete sequence scFvs, variable domains and CDRs, and provides guidance on what regions can be mutated.” July 2, 2004 Amendment & Remarks, Strohl Decl. Ex. L, at 12, ECF No. 112-12.

On September 13, 2004, the Examiner again rejected the same claims for lack of enablement in an almost identical manner. Sept. 13, 2004 Rejection, at 2. The primary difference between the first and second rejection was that the Examiner now referred to the enabled portions of the scFvs embodied by SEQ ID NOs 82, 84, and 86 as “antibody fragments” rather than “antibody derivatives.” *Id.* The Examiner maintained his rejection of *antibody derivatives* comprising “chimeric antibodies, humanized antibodies, single chain antibodies, bispecific antibodies, diabodies and di-, oligo- or multimers thereof in claim 4.” *Id.*

Case 1:17-cv-00509-TBD Document 330 Filed 12/03/18 Page 15 of 31 PageID #: 23302

On October 16, 2004, the Examiner conducted a telephonic interview with the applicant. Oct. 16, 2004 Interview Summary, Strohl Decl. Ex. N, ECF No. 112-14. During that interview the Examiner “suggested that the claims be amended to recite antibody fragment thereof to substitute antibody derivative.” *Id.* at 1. Thereafter, the applicants amended their claims to implement the changes proposed in the interview by, among other things, revising the claims to recite *antibody fragments* instead of *antibody derivatives*. Dec. 13, 2004 Amendment, Strohl Decl. Ex. O, ECF No. 112-15.

Thus, *antibody derivatives* except for *antibody fragments* were disclaimed from the scope of the claims. As was made clear by the January 2 and September 13 rejections, the Examiner found enabled *antibody fragments*, that is, fragments of antibodies as defined in the specification. By way of example, in the case of the scFvs described by SEQ ID NOs 82, 84, and 86, the Examiner found enabled only the pieces of those scFvs that are naturally occurring—i.e., the V<sub>H</sub> and V<sub>L</sub> regions—and found not enabled the pieces of scFvs that contained an engineered peptide—i.e., the linker sequence. Moreover, the Examiner maintained his rejection of derivatives comprising, for example, chimeric antibodies, humanized antibodies, and bispecific antibodies. As is the case with an entire scFv, each of these structures, while possibly derived from an *antibody* as defined in column 5 of the specification, would have been understood, in view of the '590 patent, to involve some form of alteration to a naturally occurring antibody. *See* Markman 167:10–168:9 (Dr. Strohl); Prelim. Inj. Tr. 276:10–11 (Dr. Krishnaswamy testifying that “[a]nything you do to the antibody creates a derivative”). Thus, *antibody derivatives* as originally understood—i.e., everything derived from an antibody other than antibody fragments—were disclaimed from the claims, and the narrower *antibody fragments* were claimed

instead. On the face of the prosecution history, it therefore appears that the disclaimer of *antibody derivatives* included *bispecific antibodies*.

Not surprisingly, the parties agree that *antibody derivatives* were disclaimed from the scope of the '590 patent. Markman Tr. 10:4–21 (parties agreeing with the court that there is “agreement that there was a disclaimer in the prosecution as to derivatives, but there’s no agreement as to what a derivative is”). But they disagree on what that term encompasses. *Id.* At the preliminary injunction stage, Baxalta was unable to provide any meaningful guidance as to what, in fact, was disclaimed. Prelim. Inj. Tr. 32:2–23, 33:23–35:15. During the preliminary injunction hearing, Baxalta stated only that while derivatives may very well have been disclaimed during prosecution, the specification and prosecution history “tell[] us very little of the meaning.” *Id.* at 33:23–34:5.

At the Markman hearing, Baxalta finally addressed the issue directly, arguing that the disclaimer was very limited and that only two minor embodiments were surrendered, a suggestion not appearing in its earlier briefing. Baxalta’s arguments related to two amendments made during prosecution.

The first amendment related to original claims 5 and 6, which read:

5. An antibody derivative according to claim 1, wherein said antibody derivative comprises a complement [sic] determining region (CDR) peptide.
6. An antibody derivative according to claim 5, wherein said CDR peptide is a CDR3 peptide.

U.S. Patent App. No. 09/661,992, at 62. The Examiner rejected these claims as not enabled because the “specification provides no direction or guidance regarding how to produce such antibodies.” Jan. 2, 2004 Rejection, at 5. The Examiner explained that because “each of the heavy and light chain CDRs are critical in maintaining the antigen binding specificity and

affinity which is characteristic of the parent immunoglobulin” “[i]t is unlikely that antibody derivatives as defined by the claims which may contain less than the full complement of CDRs . . . have the required binding function.” *Id.* In response, and following an interview with the Examiner in which he requested that the applicants add two dependent claims regarding the CDR3 peptide, Oct. 16, 2004 Interview Summary, the applicants deleted original claims 5 and 6, and added current claims 21 and 22, which are as follows:

- 21. The antibody or antibody fragment of claim 1, wherein the antibody fragment comprises a CDR3 peptide.
- 22. The antibody or antibody fragment of claim 1, wherein the antibody fragment is a CDR3 peptide.

Dec. 13, 2004 Amendment, at 7 (numbered 30. and 31. in amendment); '590 patent, col. 104, ll. 9–12.

At the Markman hearing Baxalta was unable to explain why these amendments defined the scope of the disclaimer of *antibody derivatives*. Earlier the Examiner had expressed concern that the original claims' reference to CDR1 and CDR2 peptides was not enabled, and Baxalta opined that “the examiner wanted to be assured that that which is critical to antigen binding, the CDR3, was included in the claim,” Markman Tr. at 36:5–11. But this sheds no light on the meaning of the term *antibody derivative* or the scope of the disclaimer. All three binding sites (CDR1, CDR2, and CDR3) exist in *antibodies* as defined in the specification and under the broader definition of *antibody*. Referring specifically to the CDR3 binding sites in two dependent claims is perfectly consistent with the narrower definition of *antibody*. In short, while Baxalta agrees that there was a disclaimer of *antibody derivatives* it is unable to show that the term was somehow limited by, or defined by, the addition of the reference to the CDR3 binding site in the dependent claim.

The second amendment on which Baxalta focused was that made to original claim 7, which provided

7. An antibody derivative according to claim 6, wherein said CDR3 peptide comprises an amino acid sequence selected from the group consisting of:  
Tyr-Gly-Asn-Ser-Pro-Lys-Gly-Phe-Ala-Tyr;  
Cys-X-X-Tyr-Gly-Asn-Ser-Pro-Lys-Gly-Phe-Ala-Tyr-X-X-Cys  
Wherein  
X may be any desired amino acid;  
Tyr-Gly-Asn-Ser-Pro-Lys-Gly-Phe-Ala-Tyr;  
Asp-Gly-Gly-His-Gly-Tyr-Gly-Ser-Ser-Phe-Asp-Tyr; and  
Phe-Arg-Asn-Arg-Gly-Met-Thr-Ala-Leu-Leu-Lys-Val-Ser-Ser-Cys-Asp.

Strohl Decl. Ex. D, at 2–3. The claim language on its face does not identify a specific amino acid, or set of amino acids, that can be substituted for the variable “X,” and provides only that “X may be any desired amino acid.” *Id.* The Examiner rejected this claim as not enabled because he reasoned that “it is unpredictable if any functional activity will be shared by two antibodies having less than 100% identity over their CDR3 region.” Jan. 2, 2004 Rejection, at 5; *accord* Sept. 13, 2004 Rejection, at 3. Again following an interview with the Examiner in which “the antibody derivative and CDR3 peptide” were discussed, Oct. 16, 2004 Interview Summary, the applicants amended claim 7 (now claim 5) to address the Examiner’s concern with variability in the CDR3. Claim 5 now provides

5. A CDR3 peptide of the antibody or antibody fragment according to claim 1 consisting of an amino acid sequence selected from the group consisting of:  
Tyr-Gly-Asn-Ser-Pro-Lys-Gly-Phe-Ala-Tyr (SEQ ID NO:5); and  
Asp-Gly-Gly-His-Gly-Tyr-Gly-Ser-Ser-Phe-Asp-Tyr (SEQ ID NO:6).

Dec. 13, 2004 Amendment, at 4–5; ’590 patent, col. 101, ll. 57–63.

During the Markman hearing Baxalta argued that this amendment disclaimed “antibody derivatives comprising CDR3 peptides with variable or random amino acids as originally claimed in 7.” Markman Tr. at 36:21–37:9. But again, as was the case with the other amendment, this amendment sheds no light on the meaning of the disclaimed *antibody*

*derivatives*. It is entirely possible that there are *antibodies* and *antibody fragments*, as those terms are defined by the court, that contain a CDR3 region comprising what was claimed in both original claim 7 and amended claim 7. The specification and amended claims are perfectly consistent with the specification's definition of *antibody*.

Given Baxalta's failure to offer a plausible alternative definition of the disclaimer, the court concludes that the obvious definition—a disclaimer of antibodies altered in some significant way—should govern.

***c. The Language of the Dependent Claims as Issued Does Not Require That the Term Antibodies Standing Alone be Defined to Include Bispecific Antibodies***

Baxalta's primary argument is that adopting the narrow construction of *antibodies* is impermissible because, after the reference to *antibody derivatives* was deleted during prosecution, certain dependent claims covered structures that would be excluded under the narrower construction of the term *antibody*. In other words, Baxalta argues that including such dependent claims after the amendment (which deleted *antibody derivatives* from the claims) effectively redefines the term *antibody*. The language of the allowed claims is set forth above. Baxalta points, for example, to dependent claim 4, which claims "[a]n antibody or antibody fragment according to claim 1, wherein said antibody or antibody fragment is selected from a group consisting of a monoclonal antibody, a chimeric antibody, a humanized antibody, a single chain antibody, a bispecific antibody, a diabody, and di-, oligo-, or multimers thereof." '590 patent, col. 101, ll. 51–56. Baxalta contends that under the narrower construction of *antibody*, the humanized antibodies, chimeric antibodies, and bispecific antibodies of claims 4 and 19 would be excluded from the scope of the claims, as would the IgM and IgA antibodies of claims 3 and 20, and the artificial linker sequences of claims 7, 9, and 11. Baxalta Op. Br. 4–5; Baxalta Supp. Ltr. 1–3, ECF No. 202; Markman Tr. at 40:8–45:2 (citing Strohl Dep. Tr., Dadush Decl.

Ex. 1, ECF. No. 234-2 at 99:23–100:13; Prelim. Inj. Tr. 61:4–7). Baxalta insists that “[i]t is axiomatic that a dependent claim cannot be broader than the claim from which it depends.” *Alcon Research, Ltd. v. Apotex Inc.*, 687 F.3d 1362, 1367 (Fed. Cir. 2012); *see also* Baxalta Op. Br. 4–5; Baxalta Supp. Ltr. 1–3. It reasons that the independent claims, such as claim 1, must encompass *bispecific antibodies* and that the term *antibody* must therefore include *bispecific antibodies*.

I do not find Baxalta’s argument persuasive. To be sure, Baxalta is correct, and Genentech agrees, that at least dependent claims 4 and 19 are inconsistent with the narrower definition of the term *antibody*.<sup>10</sup> *See* Markman Tr. 143:2–19 (Genentech admission that claims 4 and 19<sup>11</sup> are inconsistent with the column 5 definition of *antibody*). The Federal Circuit has recognized that adopting a construction that excludes dependent claims from the patent scope is disfavored. *See AK Steel Corp. v. Sollac & Ugine*, 344 F.3d 1234, 1242 (Fed. Cir. 2003) (“Under the doctrine of claim differentiation, dependent claims are presumed to be of narrower scope than the independent claims from which they depend.”). But the court has also made clear that this rule of construction does not govern where the independent claims on their face are of more limited scope. *See Enzo Biochem Inc. v. Applera Corp.*, 780 F.3d 1149, 1156–57 (Fed. Cir.

---

<sup>10</sup> Other dependent claims alleged by Baxalta to be inconsistent with the narrower definition of *antibody* may well be consistent with that definition. For example, even though IgM and IgA antibodies can have more than two identical light chains and two identical heavy chains, they can also be understood as being composed of several monomers that have the structure identified in column 5. *See* Markman Tr. 112:15–23 (Dr. Almagro agreeing that “an IgM is a pentamer of a module that has two identical heavy chains and two identical light chains” and “that module meets the definition in column 5”); *id.* 114:17–23 (Dr. Almagro admitting that “some IgMs circulate as monomers in serum”); *id.* 155:24–159:11 (Dr. Strohl confirming that IgM and IgA antibodies sometimes contain two identical light chains and two identical heavy chains).

<sup>11</sup> It is apparent from context that Genentech’s counsel misspoke when he named claim 9, rather than claim 19, as being inconsistent with the column 5 definition.

2015) (claim differentiation rejected as reason to broaden scope of independent claim contrary to its plain meaning). That is the case here, for the reasons disclosed above.

In such situations, where the scope of the independent claims is clear, the Federal Circuit has held that the failure of a patentee to conform dependent claims to the scope of the independent claims results in invalidation of the inconsistent claims rather than an expansion of the independent claim. *See Regents Univ. of Cal. v. Dakocytomation Cal., Inc.*, 517 F.3d 1364, 1375–76 (Fed. Cir. 2008); *Seachange Int'l, Inc. v. C-COR, Inc.*, 413 F.3d 1361, 1369–75 (Fed. Cir. 2005); *N. Am. Vaccine, Inc. v. Am. Cyanamid Co.*, 7 F.3d 1571, 1577–78 (Fed. Cir. 1993). For instance, in *Dakocytomation*, the Federal Circuit rejected the appellants' argument that the district court's claim construction, which excluded repetitive sequences, was "incorrect in light of certain dependent claims requir[ing] inclusion of repetitive sequences." 517 F.3d at 1375. The court explained that, although there exists a presumption that dependent claims have narrower scope than the independent claims from which they depend, "[p]resumptions are rebuttable." *Id.* Thus, "while it is true that dependent claims can aid in interpreting the scope of claims from which they depend, they are only an aid to interpretation and are not conclusive." *Id.* (quoting *N. Am. Vaccine*, 7 F.3d at 1577 (brackets omitted)). The Federal Circuit emphasized that a contrary construction "dictated by the written description or the prosecution history" could overcome the presumption. *Id.* (quoting *Seachange*, 413 F.3d at 1369).

I conclude that the presumption that the independent claim here is broader than the dependent claim has been rebutted. The retention of inconsistent language in the dependent claims does not suggest that the interpretation of independent claims should depart from the original meaning of the term *antibody* as provided in column 5 of the '590 patent. *See Dakocytomation*, 517 F.3d at 1375–76 ("Here... the prosecution history overcomes the



presumption; the correct construction of ‘heterogeneous mixture’ is one that excludes repetitive sequences, notwithstanding the presence of certain dependent claims that do not exclude them.”); *Seachange*, 413 F.3d at 1375 (“The presumption attendant to claim differentiation doctrine is rebutted. The phrase ‘network for data communications’ is limited to networks in which every processor system is connected to every other processor system via direct, point-to-point, two-way channel interconnections.”); *N. Am. Vaccine*, 7 F.3d at 1577–78 (rejecting construction of independent claim based on scope of dependent claims because “[t]he dependent claim tail cannot wag the independent claim dog”). While the result of the court’s construction is that certain embodiments in the specification are no longer covered by the claims, this is not uncommon in disclaimer situations.

Finally, even with the narrowing amendment to delete *antibody derivative*, the narrower definition of *antibody* does not exclude any disclosed embodiments actually made by the inventors from the scope of the claims. As Genentech established through its expert, Dr. Strohl’s testimony, which I credit, and through cross-examination of Dr. Almagro, the patent does not disclose that the inventors of the ’590 patent ever made a bispecific antibody, a humanized antibody, or a chimeric antibody. Markman Tr. at 110:12–20 (Dr. Almagro regarding humanized and bispecific antibodies); *id.* at 179:23–180:25 (Dr. Strohl testifying that Example 13 of the ’590 patent is prophetic and therefore does not show that the inventors created a chimeric antibody within the scope of the claims). Rather, as Dr. Almagro admitted, “all of the antibodies that Baxalta made in performing the experiments set forth in [the ’590] patent would fall within the definition of column 5.” Markman Tr. 105:15–21.

Based on the foregoing, I conclude that the term *antibody* means an immunoglobulin molecule, having a specific amino acid sequence that only binds to the antigen that induced its

synthesis or very similar antigens, consisting of two identical heavy chains (H chains) and two identical light chains (L chains). Baxalta concedes that Hemlibra does not infringe the '590 patent under the court's construction of *antibody*. Prelim. Inj. Tr. 9:15–24.

## 2. *antibody fragment*

**Baxalta's proposed construction:** A portion of a molecule having a specific amino acid sequence comprising two heavy chains (H chains) and two light chains (L chains).

**Genentech's proposed construction:** A fragment of an antibody which partially or completely lacks the constant region; the term “antibody fragment” excludes all other forms of antibody derivatives.

**Court's construction:** A fragment of an antibody which partially or completely lacks the constant region; the term “antibody fragment” excludes bispecific antibodies.

Though substitution of the word *fragments* for *derivatives* preserved the applicant's claim to derivatives that are fragments, there is no assertion here that the term *fragments* included bispecific antibodies, or expanded the defined meaning of the term *antibody*. Nor is there any contention that Hemlibra is a fragment. “[T]here is no dispute between the parties that [Hemlibra], the accused product, is not a fragment. It's a full-length antibody.” Prelim. Inj. Tr. 79:10–15; *accord* Markman Tr. 122:13–21 (Dr. Almagro agreeing that Hemlibra “is a bispecific antibody,” “not a Fab” and “not a fragment”). And as Baxalta conceded during the preliminary injunction hearing, “the inclusion of the word ‘fragment’ wouldn't expand the meaning of the term ‘antibody.’” Prelim. Inj. Tr. 35:23–36:2.

Baxalta argues that under the court's definition of *antibody* any piece of an antibody can be considered an *antibody fragment*. Genentech argues instead that the term *antibody fragment* includes only “the sort of canonical antibody fragments that [a person of skill] talks about when [that person] talks about a fragment.” Markman Tr. 11:11–12. In light of the prosecution

history, Genentech contends that this sort of antibody fragment comprises a portion of an antibody which partially or completely lacks its constant region.

The specification expressly states that “antibody fragments . . . partially or completely lack the constant region.” ’590 patent, col. 6, ll. 20–21. The examples of fragments listed in the specification—“Fv, Fab, Fab’ [and] F(ab)’<sub>2</sub>”—all support this limitation. *Id.*; see also *supra* at 11–12.

In summary, the court rejects Baxalta’s proposed construction of *antibody fragment*. The Baxalta definition is unduly broad because it would include something as small as three amino acids, which Dr. Almagro agreed would not be understood by a person skilled in the art to constitute an *antibody fragment*. When asked if you “cut the last three amino acids off the constant region” and ask an antibody scientist in 1993 whether that is an antibody fragment, Dr. Almagro agreed that “[s]he’d say ‘No, that’s three amino acids.’” Markman Tr. 117:19–118:4.

Therefore, the court finds that *antibody fragment* comprises a fragment of an antibody which partially or completely lacks the constant region; the term “antibody fragment” excludes bispecific antibodies.

### 3. *bispecific antibody*

**Baxalta’s proposed construction:** An antibody that is a macromolecular, heterobifunctional cross-linker having two different binding specificities within one single molecule.

**Genentech’s proposed construction:** An antibody derivative that is an artificially engineered, macromolecular, heterobifunctional cross-linker having two different binding specificities within one single molecule; a bispecific antibody does not consist of two identical heavy chains and two identical light chains.

**Court’s construction:** An artificially engineered, macromolecular, heterobifunctional cross-linker having two different binding specificities within one single molecule; a bispecific antibody does not consist of two identical heavy chains and two identical light chains.

Case 1:17-cv-00509-TBD Document 330 Filed 12/03/18 Page 25 of 31 PageID #: 23312

The parties agree a *bispecific antibody* in the patent is a macromolecular, heterobifunctional cross-linker having two binding specificities within one single molecule, and does not consist of two identical heavy chains and two identical light chains.<sup>12</sup> Markman Tr. 38:5–16. The court construes the term *bispecific antibody* consistent with the parties' agreement. Therefore, a *bispecific antibody* is an artificially engineered, macromolecular, heterobifunctional cross-linker having two different binding specificities within one single molecule; a *bispecific antibody* does not consist of two identical heavy chains and two identical light chains.

#### 4. *isolated*

**Baxalta's proposed construction:** Essentially free from other antibodies or antibody fragments that do not bind Factor IX or Factor IXa.

**Genentech's proposed construction:** Free of molecularly non-identical antibodies or antibody fragments; all antibody molecules or antibody fragment molecules in the claimed composition are identical.

**Court's construction:** All antibody molecules or antibody fragment molecules in the claimed composition have identical amino acid sequences except for any post-translational modifications.

Construction of *isolated* appears to be relevant to the question of patent validity. As to the term *isolated*, the parties dispute the degree to which antibodies or antibody fragments that are *isolated* must be identical. The specification provides no apparent guidance as to the meaning of the term. *Isolated* is only used twice in the body of the specification, and neither reference illuminates what it means for an *antibody* or *antibody fragment* to be *isolated*. See

---

<sup>12</sup> Although Dr. Almagro agreed that bispecific antibodies generally “don’t have two identical heavy chains and two identical light chains,” he noted that “[t]here are some recent examples” to the contrary. Markman Tr. 108:11–15. Genentech clarified, and Dr. Almagro confirmed, that the recent examples Dr. Almagro referenced are bispecific antibodies developed by Genentech that “in fact, have identical chains.” *Id.* 108:16–20. But the patent’s reference to *bispecific antibodies* does not include these antibodies with identical chains.

Case 1:17-cv-00509-TBD Document 330 Filed 12/03/18 Page 26 of 31 PageID #: 23313

'590 patent, col. 32, l. 37–38 (“clones were isolated”); *id.* col. 32, ll. 57–58 (“the gene of the 198/B1 scFv was isolated from the plasmid”).

The prosecution history is also unhelpful. The Examiner added the term *isolated* to the claims during prosecution but neither explained why the change was made, nor why the change was necessary for allowance. *See* Dec. 21, 2004 Interview Summary, Cole Decl. Ex. 2, ECF No. 162-1; Notice of Allowance and Fees Due, U.S. Patent Appl. No. 09/661,992 (P.T.O. Dec. 29, 2004), ECF 202-1. Genentech argues that the term *isolated* was added to claim 1 of the '590 patent alongside the “inventors’ efforts to subclone mixed populations of hybridomas ‘to obtain homogenous [sic] cell populations.’” Genentech Op. Br. 14 (quoting '590 patent, col. 11, l. 53). Because such subcloning was performed until each hybridoma population “produce[d] the same FIX/FIXa binding antibody,” '590 Patent, col. 12 l. 19–21, Genentech contends that antibodies or antibody fragments which are *isolated* must be identical.

The sole expert testimony regarding this term comes from Genentech’s expert, Dr. Strohl. Dr. Strohl explained that *isolated* in context of the specification’s description of dilution subcloning would be understood to limit the antibodies and antibody fragments of claim 1 to those that have an “identical amino acid sequence” but not necessarily an identical glycosylation pattern. Strohl Claim Const. Decl. ¶¶ 124–27. Dr. Strohl also admitted that slight differences in the amino acid sequences of antibodies may arise due to a process called post-translational modification. Strohl Dep. Tr. 168:4–20, ECF No. 159-1.

Baxalta offers no testimony, evidence, or argument to the contrary. Based on the content of the specification, Baxalta contends only that the term *isolated* cannot require identicalness because such a construction ignores the contrast between the terms *isolated* and *purified* in the claims and specification of the patent. Baxalta Resp. Br. 2–4, ECF No. 234 (citing '590 Patent,

Case 1:17-cv-00509-TBD Document 330 Filed 12/03/18 Page 27 of 31 PageID #: 23314

cl. 17; *id.* col. 9 ll. 11–15; *id.* col. 13, ll. 16–58). Baxalta argues that because the patent discusses “purifying” an antibody after it is “isolated,” antibodies that are *isolated* cannot all possibly be identical, because were that the case, purification would be unnecessary. *Id.* I disagree with this understanding of the patent. Purification is meaningful even under Genentech’s interpretation since the reference to purification in the patent describes a solution containing antibodies and the cells from which they are derived (hybridomas), and purification eliminates those cells from the final product. *See, e.g.,* ’590 patent, col. 13, ll. 16–42.

Baxalta further points to a case, *Morphosys AG v. Janssen Biotech, Inc.*, No. 16-221-LPS, 2017 WL 4769368, at \*4 (D. Del. Oct. 23, 2017), in which *isolated* was construed to mean “essentially free from antibodies that do not bind to CD38.” The court’s construction in *Morphosys* is not relevant, as the case dealt with an entirely different patent. *See Monsanto Co. v. Bayer Bioscience N.V.*, 363 F.3d 1235, 1244 (Fed. Cir. 2004) (stating that “similar terms can have different meanings in different patents depending on the specifics of each patent”).

Thus, Genentech’s construction most clearly corresponds to how *isolated* was used in the patent, taking into account the caveats raised by Dr. Strohl regarding glycosylation and post-translational modification. Accordingly, I find that the term *isolated* requires that all antibody molecules or antibody fragment molecules in the claimed composition have identical amino acid sequences except for any post-translational modifications.

#### **5. binds Factor IX or Factor IXa and increases**

**Baxalta’s proposed construction:** “and” has its plain and ordinary meaning.

**Genentech’s proposed construction:** The increase in procoagulant activity of Factor IXa is caused only by the binding of the antibody or antibody fragment to Factor IX/IXa.

**Court’s construction:** Binding to Factor IX need not be the sole cause of the increase in procoagulant activity.

This court's construction of *binds Factor IX or Factor IXa and increases* appears to be relevant to infringement. As to this term, the parties dispute only the degree of causation required between the binding of the inventive antibodies or antibody fragments to Factor IX/IXa and the resultant increase in procoagulant activity. Genentech contends that the increase in procoagulant activity must be caused only by the binding of the inventive antibodies or antibody fragments with Factor IX/IXa. Genentech Op. Br. 17–19. Baxalta, on the other hand, argues that sole causation is not required. Baxalta Op. Br. at 14–15.

Genentech cites various portions of the specification in support of its construction. The abstract provides “[a]n antibody or antibody derivative against factor IX/activated factor IX (FIXa) which increases the procoagulant activity of FIXa.” ’590 patent, Abstract. The title of the patent is “Factor IX/Factor IXa Activating Antibodies and Antibody Derivatives.” *Id.* col. 1. The Summary of the Invention states that the “object of the present invention to provide a preparation for the treatment of blood coagulation disorders” is “achieved through the use of antibodies or antibody derivatives against factor IX/factor IXa which have factor VIIIa-cofactor activity or factor IXa-activating activity and lead to an increase in the procoagulant activity of factor IXa.” *Id.* col. 2, ll. 25–33. The specification states that “hybridomas are selected with a view to the fact that the antibodies and antibody derivatives in the supernatants of the hybridoma cells bind to factor IX/factor IXa and cause an increase of the procoagulant activity of factor IXa.” *Id.* col. 8, ll. 18–21. Though these portions of the specification certainly imply that binding to Factor IX/IXa plays a role in causing an increase in procoagulant activity, they do not suggest *sole* causation as Genentech contends.

Because nothing in the intrinsic record suggests otherwise, I find this term unambiguous and conclude that *and* in the term *binds Factor IX or Factor IXa and increases* means that binding to Factor IX need not be the sole cause of the increase in procoagulant activity.

**6. *the procoagulant activity of Factor IXa and increases the procoagulant activity of Factor IXa***

**Baxalta's proposed construction:** The rate of clot formation promoted by Factor IXa.

**Genentech's proposed construction:** The ability of Factor IXa to activate Factor X to Factor Xa by any amount as determined by any assay used to measure Factor VIII-like activity.

**Court's construction:** The ability of Factor IXa to activate Factor X to Factor Xa by any amount as determined by any assay used to measure Factor VIII-like activity.

The court's construction of this term appears to be relevant to the question of patent validity. The parties' final dispute relates to the term *increases the procoagulant activity of Factor IXa*. This construction raises the question of how the patent claims instruct a person of skill in the art to measure the procoagulant effect of the inventive antibodies and antibody fragments. Baxalta proposes a construction that limits the assessment of procoagulant activity to tests that measure the rate of clot formation—e.g., by use of Activated Partial Thromboplastin Time (“aPTT”) assays, Baxalta Op. Br. at 16–19. Genentech, on the other hand, argues that any prior art test for measuring Factor VIII-like activity, including clotting-time and chromogenic assays, can be used—e.g., aPTT assays and chromogenic assays like COATEST VIII(C) and Immunochrom, Genentech Op. Br. at 16; Markman Tr. 223:6–15; *id.* at 224:14–17.

I conclude that the specification provides clear guidance that any prior art assay that can measure Factor VIII-like activity may be used. The patent twice expressly states that any method for determining Factor VIII-like activity may be used to measure procoagulant activity: In column 8 the patent provides that “[t]he increase in the procoagulant activity may, e.g., be



Case 1:17-cv-00509-TBD Document 330 Filed 12/03/18 Page 30 of 31 PageID #: 23317

proven by assaying methods as known from the prior art for the measurement of factor VIII-like activity, e.g. chromogenic assays.” ’590 patent, col. 8, ll. 21–25. And in column 9 the patent states

The following methods may be used as the test methods to show that the antibodies and antibody derivatives of the present invention bind to factor IX/factor IXa, increase the procoagulant activity of factor IXa or have factor VIII-like activity: the one step coagulation test . . . or the chromogenic tests, such as COATEST VIII:C® (Chromogenix) or Immunochrom (IMMUNO). In principle, all the methods used for determining factor VIII activity may be used.

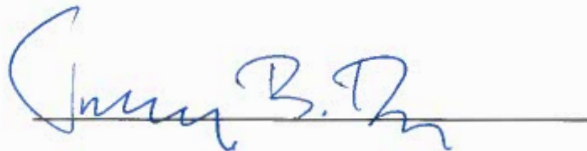
*Id.* at col. 9, ll. 14–23. The patent reiterates this point in Example 5, where the specification states that “[f]actor VIII activity is usually determined with a chromogenic assay and/or an APTT-based clotting assay” and that “[b]oth types of assays rely on FVIIIa/FIXa-mediated factor Xa generation.” *Id.* col. 15, ll. 10–13.

There is no support for Baxalta’s contrary position that only clotting-time assays are permissible. Baxalta’s primary argument is that the ’590 patent in certain places refers to clotting time assays alone as a measure of procoagulant activity. For example, Baxalta points to column 17 of the patent, which states that “[t]here is a clear dose-dependent reduction of the clotting time in samples supplemented with antibody 193/AD3” and that such “results imply that [the] antibody . . . is procoagulant in the presence of FIXa.” *Id.* col. 17, ll. 35–38. Baxalta also notes that column 29 of the patent states that a particular peptide “becomes procoagulant as indicated by the reduced clotting time” and that column 23 similarly states that certain peptides “did not give any reduction in the clotting time indicating that they lack procoagulant activity.” *Id.* at col. 29, ll. 36–40; *id.* at col. 23, l. 66–col. 24, l. 2. The patent’s reference to the use of clotting-time assays to measure procoagulant activity hardly excludes other possible methods of measurement, particularly where other parts of the specification state that “assaying methods as known from the prior art” may be used. *Id.* at col. 8, l. 21–25.

Baxalta also relies on the statement in column 9 of the patent that the inventive antibodies and antibody fragments “increase the procoagulant activity of factor IXa or have factor VIII-like activity.” *Id.* at col. 9, ll. 17–18. It contends that the use of “or” implies a distinction between procoagulant activity and Factor VIII-like activity. Baxalta Resp. Br. at 8–12. Baxalta argues that Example 9 illustrates the same distinction. *See* ’590 patent, col. 18, ll. 22–67. It follows, says Baxalta, that the term “procoagulant activity” and “factor VIII-like activity” are mutually exclusive, so that the chromogenic assay used to determine factor VIII-like activity cannot be used to determine procoagulant activity. The premise of this argument is simply incorrect. Procoagulant activity and Factor VIII-like activity are not distinct terms, but rather are overlapping.

Accordingly, I conclude that the term *increases the procoagulant activity of Factor IXa* means the ability of Factor IXa to activate Factor X to Factor Xa by any amount as determined by any assay used to measure Factor VIII-like activity.

**IT IS SO ORDERED** this 3 day of December, 2018.

A handwritten signature in blue ink, appearing to read "Timothy B. Dyk", is written over a horizontal line.

Honorable Timothy B. Dyk  
United States Circuit Judge, sitting by designation

**IN THE UNITED STATES DISTRICT COURT  
FOR THE DISTRICT OF DELAWARE**

BAXALTA INCORPORATED and BAXALTA  
GMBH,

Plaintiffs,

v.

GENENTECH, INC. and CHUGAI  
PHARMACEUTICAL CO., LTD.,

Defendants.

C.A. No. 17-509-TBD

**STIPULATION AND FINAL JUDGMENT**

WHEREAS, Plaintiffs Baxalta Incorporated and Baxalta GmbH (collectively, “Plaintiffs” or “Baxalta”) brought this action against Genentech, Inc. (“Genentech”) and Chugai Pharmaceutical Co., Ltd. (“Chugai”) alleging infringement of certain claims of U.S. Patent No. 7,033,590 (“the ’590 patent”);

WHEREAS, on September 19, 2018, the Court entered an order dismissing Chugai from this action (D.I. 293), leaving Genentech as the sole remaining defendant;

WHEREAS, on December 3, 2018 the Court entered an Opinion and Order (D.I. 330) construing disputed terms in the asserted claims of the ’590 patent, including, among other terms, the terms “antibody” “antibody fragment”, “antibody derivative” and “increasing the procoagulant activity...”;

WHEREAS, Baxalta concedes that HEMLIBRA® (emicizumab-kxwh), the only accused product in this action, does not infringe the asserted claims of the ’590 patent under the Court’s

construction of “antibody”, “antibody fragment”, or “antibody derivative” and Baxalta disagrees with and reserves all rights as to the Court’s construction of “procoagulant activity”;

WHEREAS, in exchange for Baxalta’s agreement to stipulate to a judgment of non-infringement without waiver of the right to appeal from that judgment based on the Court’s claim construction Order, Genentech agrees to stipulate to the dismissal without prejudice of its counterclaims for a declaratory judgment of non-infringement and a declaratory judgment of invalidity (*see* D.I. 240) without waiver of any argument or defenses;

WHEREAS, the Court has issued a letter rogatory (D.I. 326) that has been submitted to the United States Department of State for the testimony of Dr. Randolph Kerschbaumer, one of the inventors on the ’590 patent; and

WHEREAS, in order to promote judicial efficiency and conserve litigation costs, the parties desire to extend the deadlines for Genentech to seek costs and attorneys’ fees until the end of any appellate proceedings;

IT IS STIPULATED and ORDERED THAT:

1. Final judgment is entered in favor of Genentech and against Baxalta on all claims asserted in the operative complaint (the Amended Complaint for Patent Infringement, D.I. 239);
2. Genentech’s counterclaims for a declaratory judgment of non-infringement and a declaratory judgment of invalidity are dismissed without prejudice;
3. The Clerk of the Court is directed to enter final judgment in accordance with this stipulation;
4. Nothing in this stipulation and judgment will affect Genentech’s ability to take the deposition of Dr. Randolph Kerschbaumer while any appeal from this judgment is pending if the

requirements of Fed. R. Civ. P. 27(b) are satisfied, and if Genentech moves under Fed. R. Civ. P.

27(b) to take that deposition during such an appeal, Baxalta will not oppose that motion;

5. The deadlines for submission of a bill of costs and any motions for fees, including any motion under 35 U.S.C. § 285, are extended until (a) 21 days after the Federal Circuit issues its mandate, if Baxalta appeals from this judgment, or (b) 21 days after Baxalta's deadline to file notice of appeal, if Baxalta does not appeal from this judgment, or (c) 21 days after Baxalta discontinues any appeal from this judgment; and

6. This stipulated final judgment is without prejudice to either party's rights to appeal.

Date: January 31, 2019

Respectfully submitted,

/s/ Amy M. Dudash

Amy M. Dudash (Bar No. 5741)  
Jody C. Barillare (Bar No. 5107)  
**MORGAN LEWIS & BOCKIUS LLP**  
1007 N. Orange St. Suite 501  
Wilmington, DE 19801  
Tel. 302.574.3000  
jody.barillare@morganlewis.com  
amy.dudash@morganlewis.com

Margaret A. McGreal  
Julie S. Goldemberg  
**MORGAN, LEWIS & BOCKIUS LLP**  
1701 Market Street  
Philadelphia, PA 19103  
Tel. 215.963.5000  
margaret.mcgreal@morganlewis.com  
julie.goldemberg@morganlewis.com

Michael J. Abernathy  
Sanjay K. Murthy  
Jason C. White  
Christopher J. Betti  
Amanda S. Williamson  
Jennifer M. Dienes  
Jesse T. Dyer

/s/ Steven J. Balick

Steven J. Balick (#2114)  
Andrew C. Mayo (#5207)  
**ASHBY & GEDDES**  
500 Delaware Avenue  
P.O. Box 1150  
Wilmington, DE 19899  
(302) 654-1888  
sbalick@ashbygeddes.com  
amayo@ashbygeddes.com

Kenneth A. Gallo  
Nicholas Groombridge  
Eric Alan Stone  
Jennifer Gordon, Ph.D.  
David Cole  
**PAUL, WEISS, RIFKIND, WHARTON & GARRISON LLP**  
2001 K Street, NW  
Washington, D.C. 20006-1047  
(202) 223-7300  
1285 Avenue of the Americas  
New York, NY 10019-6064  
(212) 373-3000  
kgallo@paulweiss.com  
ngroombridge@paulweiss.com

**IN THE UNITED STATES DISTRICT COURT  
FOR THE DISTRICT OF DELAWARE**

BAXALTA INCORPORATED and BAXALTA  
GMBH,

Plaintiffs,

v.

GENENTECH, INC. and CHUGAI  
PHARMACEUTICAL CO., LTD.,

Defendants.

C.A. No. 17-509-TBD

**STIPULATION AND FINAL JUDGMENT**

WHEREAS, Plaintiffs Baxalta Incorporated and Baxalta GmbH (collectively, “Plaintiffs” or “Baxalta”) brought this action against Genentech, Inc. (“Genentech”) and Chugai Pharmaceutical Co., Ltd. (“Chugai”) alleging infringement of certain claims of U.S. Patent No. 7,033,590 (“the ‘590 patent”);

WHEREAS, on September 19, 2018, the Court entered an order dismissing Chugai from this action (D.I. 293), leaving Genentech as the sole remaining defendant;

WHEREAS, on December 3, 2018 the Court entered an Opinion and Order (D.I. 330) construing disputed terms in the asserted claims of the ‘590 patent, including, among other terms, the terms “antibody” “antibody fragment”, “antibody derivative” and “increasing the procoagulant activity...”;

WHEREAS, Baxalta concedes that HEMLIBRA® (emicizumab-kxwh), the only accused product in this action, does not infringe the asserted claims of the ‘590 patent under the Court’s

construction of “antibody”, “antibody fragment”, or “antibody derivative” and Baxalta disagrees with and reserves all rights as to the Court’s construction of “procoagulant activity”;

WHEREAS, in exchange for Baxalta’s agreement to stipulate to a judgment of non-infringement without waiver of the right to appeal from that judgment based on the Court’s claim construction Order, Genentech agrees to stipulate to the dismissal without prejudice of its counterclaims for a declaratory judgment of non-infringement and a declaratory judgment of invalidity (*see* D.I. 240) without waiver of any argument or defenses;

WHEREAS, the Court has issued a letter rogatory (D.I. 326) that has been submitted to the United States Department of State for the testimony of Dr. Randolph Kerschbaumer, one of the inventors on the ’590 patent; and

WHEREAS, in order to promote judicial efficiency and conserve litigation costs, the parties desire to extend the deadlines for Genentech to seek costs and attorneys’ fees until the end of any appellate proceedings;

IT IS STIPULATED and ORDERED THAT:

1. Final judgment is entered in favor of Genentech and against Baxalta on all claims asserted in the operative complaint (the Amended Complaint for Patent Infringement, D.I. 239);
2. Genentech’s counterclaims for a declaratory judgment of non-infringement and a declaratory judgment of invalidity are dismissed without prejudice;
3. The Clerk of the Court is directed to enter final judgment in accordance with this stipulation;
4. Nothing in this stipulation and judgment will affect Genentech’s ability to take the deposition of Dr. Randolph Kerschbaumer while any appeal from this judgment is pending if the

requirements of Fed. R. Civ. P. 27(b) are satisfied, and if Genentech moves under Fed. R. Civ. P.

27(b) to take that deposition during such an appeal, Baxalta will not oppose that motion;

5. The deadlines for submission of a bill of costs and any motions for fees, including any motion under 35 U.S.C. § 285, are extended until (a) 21 days after the Federal Circuit issues its mandate, if Baxalta appeals from this judgment, or (b) 21 days after Baxalta's deadline to file notice of appeal, if Baxalta does not appeal from this judgment, or (c) 21 days after Baxalta discontinues any appeal from this judgment; and

6. This stipulated final judgment is without prejudice to either party's rights to appeal.

Date: January 31, 2019

Respectfully submitted,

/s/ Amy M. Dudash

Amy M. Dudash (Bar No. 5741)  
Jody C. Barillare (Bar No. 5107)  
**MORGAN LEWIS & BOCKIUS LLP**  
1007 N. Orange St. Suite 501  
Wilmington, DE 19801  
Tel. 302.574.3000  
jody.barillare@morganlewis.com  
amy.dudash@morganlewis.com

Margaret A. McGreal  
Julie S. Goldemberg  
**MORGAN, LEWIS & BOCKIUS LLP**  
1701 Market Street  
Philadelphia, PA 19103  
Tel. 215.963.5000  
margaret.mcgreall@morganlewis.com  
julie.goldemberg@morganlewis.com

Michael J. Abernathy  
Sanjay K. Murthy  
Jason C. White  
Christopher J. Betti  
Amanda S. Williamson  
Jennifer M. Dienes  
Jesse T. Dyer

/s/ Steven J. Balick

Steven J. Balick (#2114)  
Andrew C. Mayo (#5207)  
**ASHBY & GEDDES**  
500 Delaware Avenue  
P.O. Box 1150  
Wilmington, DE 19899  
(302) 654-1888  
sbalick@ashbygeddes.com  
amayo@ashbygeddes.com

Kenneth A. Gallo  
Nicholas Groombridge  
Eric Alan Stone  
Jennifer Gordon, Ph.D.  
David Cole  
**PAUL, WEISS, RIFKIND, WHARTON &  
GARRISON LLP**  
2001 K Street, NW  
Washington, D.C. 20006-1047  
(202) 223-7300  
1285 Avenue of the Americas  
New York, NY 10019-6064  
(212) 373-3000  
kgallo@paulweiss.com  
ngroombridge@paulweiss.com



**CONFIDENTIAL MATERIAL OMITTED  
FROM THE FOLLOWING PAGES:**

Appx16215-16219, Appx16230, Appx16242, Appx16249-16252, Appx16261, Appx16399, Appx16413-16422, Appx16431-16440, Appx16448-16452, Appx16514-16651, Appx16699, Appx16818, Appx16828-16892, Appx16971, Appx16974-16975, Appx16991-16994, Appx17275, Appx17279-17280, Appx17298, Appx17313-17315, Appx17339-17341, Appx17350-17353, Appx17411, Appx17435-17436, Appx17451-17453, Appx17466-17468, Appx17599-17600, Appx17607-17608, Appx17668-17671, Appx17684, Appx17711-17712, Appx18164, Appx18184-18186, Appx18202-18207, Appx18215-18217, Appx18248-18254, Appx18418-18426, Appx18575, Appx18615-18628, Appx18645, Appx18754-18755, Appx18836, Appx18843-18856, Appx18861-18875, Appx19021-19023, Appx19121-19122, Appx19132-19135, Appx19141-19142, Appx19147-19148, Appx19151, Appx19163, Appx19176, Appx19179, Appx19186-19192, Appx19197-19198, Appx19205-19226, Appx19256, Appx19321-19322, Appx19337, Appx19387-19398, Appx19519-19520, Appx19524-19525, Appx19530-19533, Appx19539-19545, Appx19670-19723, Appx19776, Appx19807, Appx20329, Appx20335

Page 1

UNITED STATES DISTRICT COURT  
FOR THE DISTRICT OF DELAWARE

-----x  
BAXALTA INC., et al., :  
:  
Plaintiffs, :  
:  
v. : No. 17-509-TBD  
:  
GENENTECH, INC., et al., :  
:  
Defendants. :  
-----x

717 Madison Place, NW.  
Washington, D.C.  
Friday, November 19, 2021

The HEARING in the above-entitled matter  
was convened at 10:00 a.m. pursuant to notice.

BEFORE:

JUDGE TIMOTHY B. DYK

Page 8

1 MR. STONE: Your Honor, we have some,  
2 that would be only the first time I do that. We  
3 have some slides that everyone will not refer to  
4 but we wanted to have them in case we do. May I  
5 hand those up?

6 JUDGE DYK: Yeah.

7 MR. STONE: I have one copy here for  
8 Your Honor and I believe a copy for the court  
9 clerk if you want to come get them. And with the  
10 Court's permission I'll take off my mask.

11 JUDGE DYK: Yes.

12 MR. STONE: Thank you. May it please  
13 the Court, Your Honor, Eric Stone for Genentech.  
14 The Court should grant summary judgement under  
15 Section 112 because no reasonable jury could find  
16 that the 590 patent provides adequate written  
17 description or if it enables the broad scope of  
18 the claim genera. In the briefing we identify six  
19 reasons why that's true. I'd like to start by  
20 uniting them into a common core. The problem here  
21 at battle is that the 590 patent is functional at  
22 the point of novelty. Taking the evidence into

Page 9

1 the right most favorable to Baxalta as we have  
2 tried to do on this motion it's inventors  
3 discovered that some antibodies that bind Factor 9  
4 or 9A increase the procoagulant activity of Factor  
5 9A. We'd accept on this motion that they made 11  
6 such antibodies. We all also agree that all of  
7 them, all 11, are mirroring. They come from a  
8 mouse. They're monospecific. They bind only  
9 Factor 9 and they're IGG or IGM type antibodies.

10 JUDGE DYK: What's the relationship  
11 between the 11 and the 10 to positive cell ones?

12 MR. STONE: There's overlap between  
13 them. Some, there are some that are described in  
14 the patent but not deposited and some that are  
15 deposited but not otherwise mentioned in the  
16 patent. You end up getting to 11 if you add them  
17 together. It's a little more complicated because  
18 it turns out two of the deposit antibodies are the  
19 same antibody and so it becomes difficult to just  
20 read the patent and get there. It's actually  
21 taken some work to get to an agreement on 11  
22 because there's overlap between what's deposited

Page 10

1 and some of the deposited antibodies are actually  
2 the same antibody. I don't, it's not clear  
3 whether and it seems likely that they didn't know  
4 that at the time they deposited them.

5 JUDGE DYK: So, if the deposits are cell  
6 line deposits so does each cell line only produce  
7 a single antibody?

8 MR. STONE: That is a dispute of fact  
9 between the parties but on this motion we're  
10 accepting yes.

11 JUDGE DYK: Okay.

12 MR. STONE: It, it is also undisputed  
13 that there are no common structural features  
14 across those antibodies. There is no disclosed  
15 mechanism of action. They don't know why they  
16 work and more to the point, they don't know why  
17 the ones that don't work don't work and there's no  
18 disclosed binding site on Factor 9 that they bind  
19 to. From that premise, they break, state down a  
20 very broad generous claim which as the Court knows  
21 has two structural requirements. It needs to be  
22 an antibody or a fragment and it needs to be

Page 47

1 MR. PETERSON: And to be clear, Your  
2 Honor, we didn't invent this test for the  
3 representiveness of the antibodies. We took this  
4 test directly from the AbbVie decision. The  
5 difference between our case and AbbVie is that  
6 AbbVie's antibodies although numerous were all  
7 very similar, were all in a very narrow corner of  
8 the claimed geneious and the accused product was  
9 very dissimilar to any of the disclosed antibodies  
10 so in AbbVie those disclosed antibodies did not  
11 reflect the full structural versa. In this case,  
12 we have 11 amino acid sequences and other  
13 attributes analyzed by our expert who looked at  
14 them and said these are very diverse sequences.  
15 They are very different from each other.

16 JUDGE DYK: But there was no comparison  
17 to the ones that didn't exhibit procoagulant  
18 activity.

19 MR. PETERSON: That's correct, Your  
20 Honor, and we don't see the purpose of this test  
21 as being to identify what is within and what is  
22 without the geneious. That's a question of

Page 48

1 enablement. Written description as Ariad has  
2 always explained it, as Ariad's progeny explained  
3 it, is about a reasonable number of species and  
4 variety of species that are within the scope of  
5 the geneious. It's proving that you have not  
6 simply as in Eli Lilly invent a rat insulin and  
7 tried to claim all vertebrae and all mammalian but  
8 have actually invented a sufficient number of  
9 species throughout the scope of the geneious to  
10 support a claim and show possession to those with  
11 skill in the art to the geneious as a whole.

12 JUDGE DYK: Well what about the fact  
13 that all of, there are 11 examples, right, and all  
14 of the examples are monospecific?

15 MR. PETERSON: Yes, Your Honor.

16 JUDGE DYK: Yeah, so how does that  
17 provide written, this written support for  
18 bispecific antibodies?

19 MR. PETERSON: So the portion of the  
20 antibody that was analyzed in AbbVie and the  
21 portion of the antibody that was analyzed by our  
22 expert was just the variable region of the

Page 49

1 antibody. This is where looking at my slides may  
2 actually be helpful because this is the point that  
3 my slides were intended to illustrate. And, and  
4 I'll give you the, the cliff notes, which is that  
5 this sort of antibody engineering, taking an  
6 antibody in one format and engineering it into  
7 another isn't unpredictable. That's a predictable  
8 art according to our experts and what makes it  
9 possible is that--

10 JUDGE DYK: Predictable, what's  
11 predictable, to, to engineer a monospecific  
12 antibody and turn it into a bispecific antibody?

13 MR. PETERSON: Yes, Your Honor, because  
14 the part of the antibody that is responsible for  
15 doing the work is the variable region. It is the  
16 variable region that is responsible for the  
17 binding affinity and specificity. It's the  
18 variable region that's responsible for this  
19 claimed procoagulant activity. So when in AbbVie  
20 when our expert looked at these disclosed  
21 antibodies what he looked at were the variable  
22 regions of the antibodies and that's what allows



Page 50

1 this antibody engineering. So if you look at the  
2 first slide you see what I think of just the mind  
3 run antibody. You've seen this many times I'm  
4 sure. You have the variable regions in red, you  
5 have the constant regions in gray. You can take  
6 that, turn it into an IGE, a different isotype.  
7 That's the second slide. You'll see the variable  
8 regions are unchanged and so we expect predictably  
9 that this is going to have the same binding  
10 specificity and exhibit the same activity. When  
11 you make a chimeric antibody you simply take the  
12 variable regions from the mouse, from the  
13 mirroring antibody, put them on the constant  
14 regions of the human antibody but because you have  
15 the variable regions you expect the same results.

16 JUDGE DYK: Well what about the fact  
17 here that the bispecific antibody for one of the  
18 light chains binds Factor 10?

19 MR. PETERSON: And, Your Honor, what we  
20 would say is that's not part of the claimed  
21 invention. The claimed invention is about the  
22 bispecific antibody with the arm that binds Factor

Page 51

1 9, 9A and that arm that exhibits the procoagulant  
2 activity on Factor 9, 9A, and it's not necessary  
3 to enable or to fully describe even the portions  
4 of the, portions of this composition that are not  
5 part of the claims. The answer is if you would  
6 like to make a bispecific antibody you can take  
7 one of these IGG antibodies that binds to any  
8 other antigen, cut it in half, put them together  
9 and predictably without undue experimentation have  
10 a bispecific antibody that has your Factor 9, 9A  
11 arm and also has another arm as well. So the  
12 testimony of our expert at least is that you don't  
13 need to be told in order to practice this claim  
14 what the other arm should be. You simply need to  
15 be told here's what one of the arms should be and  
16 then you can choose any other arm that you would  
17 like to put on there. Now it sounds like there  
18 may be a dispute of fact as to whether those with  
19 skill in the art would actually expect that to  
20 work. Our experts certainly think, one was still  
21 in the art at the time, would expect that  
22 predictably to work. Their experts may testify

Page 52

1 otherwise. That's an issue for the jury. The,  
2 the point here with this engineering --

3 JUDGE DYK: So, so there would be no  
4 infringement here if HemLibra procoagulant  
5 activity resulted from the Factor 10 binding and  
6 not from the fact that it binds, the other chain  
7 binds to Factor 9A?

8 MR. PETERSON: Your Honor, I do want to  
9 be careful there. My, my understanding is that  
10 the claim is that there must be procoagulant  
11 activity from the Factor 9, 9A arm.

12 JUDGE DYK: So there would be no  
13 infringement if the procoagulant activity came  
14 from the binding to Factor 10?

15 MR. PETERSON: If the only procoagulant  
16 activity were, came from the binding of Factor 10  
17 and there was no procoagulant activity as a result  
18 of the Factor 9, 9A arm, my understanding is that  
19 there would be no infringement.

20 JUDGE DYK: Okay.

21 MR. PETERSON: And, Your Honor, just to  
22 close the book on the slides, I did want to show

Page 53

1 how they were disclosed. If you turn to the  
2 second to last slide, you'll look at slide seven  
3 and this is one of the amino acid sequences that  
4 is actually disclosed in the specification itself.  
5 This is the amino acid sequence disclosed in  
6 Figure 14. Now this is actually not a, an IGG  
7 antibody, the type that we've been discussing.  
8 It's actually a single chain variable fragment,  
9 but what Figure 14 tells us is the top half of  
10 that is the amino acid sequence for the variable  
11 heavy chain. The bottom half of that is the amino  
12 acid sequence for the variable light chain. The  
13 gray in the middle is the linker sequence. But if  
14 one of skill in the art were to take this SCFC and  
15 using technology recombine it into an IGG they  
16 would know that that IGG would have exactly the  
17 same amino acid sequences because those variable  
18 regions aren't changed as a part of this process.  
19 Now to be clear, humanization is slightly  
20 different. You're not using the entire variable  
21 region, you're using the complimentary determining  
22 region, which is the portion of the variable

Page 54

1 region that is most responsible for the binding  
2 but these are predictable techniques and these are  
3 why in the view of our experts the antibody format  
4 is, is simply a red herring because to those with  
5 skill in the art when you possess these amino acid  
6 sequences for the variable regions you're showing  
7 that you have possession of these same variable  
8 regions, this same functionality in any sort of  
9 format. My friend talked about the AbbVie  
10 decision. I, I think that's helpful to us.  
11 There's a line from AbbVie where it was talking  
12 about that the patents didn't describe species  
13 representative of antibodies that are structurally  
14 similar to Stelara, that was the accused product.  
15 And that paragraph closes there's also no evidence  
16 to show whether one of skill in the art could make  
17 predictable changes to the described antibodies to  
18 arrive at other types of antibodies such as  
19 Stelara. Here when you're talking about the  
20 different antibody formats, the testimony of our  
21 experts at least is that you can make predictable  
22 changes to the antibody format and arrive at a

Page 55

1 different format that exhibits the same  
2 functionality. So possessing representative  
3 species of these variable regions that reflect the  
4 full structural diversity, in fact, some of them  
5 are far closer to emicizumab than they are to each  
6 other. These are very diverse disclosed species,  
7 shows that you have that same possession across  
8 all of the different antibody formats.

9 JUDGE DYK: But none of the 11 examples  
10 in the patent have therapeutic benefit, right?

11 MR. PETERSON: None of them were  
12 developed into a therapeutic product. The  
13 testimony of one of our experts, Dr. Krishna  
14 Swami, is that he thinks any procoagulant activity  
15 would have at least some therapeutic benefit,  
16 anything? It's unclear. He suggests that even if  
17 you're not completely solving the problem you  
18 could be moving a hemophilia patient from one  
19 category to another. But I think the key point as  
20 I see it is that there's no suggestion that  
21 antibodies with therapeutic benefit would somehow  
22 fall structurally outside the set of disclosed

Page 56

1     antibodies. They are some, they are somehow not  
2     being represented by this --

3             JUDGE DYK: But, but if someone says I,  
4     I'd like to get an antibody that has therapeutic  
5     benefit, this, this patent doesn't tell you how to  
6     do that, does it?

7             MR. PETERSON: Your Honor, I'd suggest  
8     there's a lot to therapeutic benefit of a drug  
9     other than simply the degree of procoagulant  
10    activity but I'll admit the patent doesn't  
11    specifically say if you'd like to achieve a  
12    certain degree of procoagulant activity. Now I  
13    think my friend's enablement arguments there were  
14    based on this idea of I'll say two things. One is  
15    this MagSil idea and that is a principle that we  
16    see applying --

17            JUDGE DYK: I'm sorry, the what?

18            MR. PETERSON: MagSil, the case.

19            JUDGE DYK: What's, a case?

20            MR. PETERSON: Sorry. Yes, Your Honor,  
21    it's, the case is MagSil versus Hitachi. It's a  
22    2012 case. And this is the one where he talked

Page 77

1 the -- on that assumption that they're assuming  
2 that your witnesses are correct? What are the  
3 factual disputes?

4 MR. PETERSON: If they're assuming that  
5 our witnesses are correct, there are no factual  
6 disputes. But the testimony of our witnesses is  
7 that one of skill in the art is enabled to  
8 practice to full scope including each of the  
9 antibody formats that's listed in the claims.

10 JUDGE DYK: Okay. But that sounds like  
11 a legal question, right?

12 MR. PETERSON: It is but it's about the  
13 quantity of experimentation underneath that. And  
14 I'm sorry if I stated it in legal terms. Each of  
15 those that are skilled in the arts said to them,  
16 it would be a routine process. It might be time  
17 consuming as in Wands. There might be a large  
18 amount of screening.

19 Wands itself was a case in which you had  
20 to screen the results of a hybridoma process.  
21 There might be a large amount of quantity but the  
22 experimentation would be routine and not undue.



Page 78

1 JUDGE DYK: Okay. Anything else beyond  
2 that?

3 MR. PETERSON: No, Your Honor. Thank  
4 you.

5 JUDGE DYK: Why don't we take a  
6 10-minute break? According to my clock here, it's  
7 20 after. We'll resume at 12:30 or 11:30, I  
8 guess. This clock hasn't been reset Standard  
9 Time.

10 (Recess)

11 JUDGE DYK: Be seated please. Okay.  
12 Let's hear again from Mr. Stone?

13 MR. STONE: Thank you, Your Honor. I'd  
14 like to start out by answering the Court's factual  
15 question from the opening argument about how many  
16 antibodies they made as best we can tell?

17 The patent tells us that there were  
18 about 1,000 per fusion. A fusion is the joining  
19 of the two types of cells together. And that  
20 there were four fusions, which gets us to at least  
21 4,000 that they screened to come to the 11. In  
22 the paper that the lead inventor wrote some memory

Page 79

1 of years later, he said it was 5,280. But between  
2 that and the 4,000 in the patent that seems like  
3 the best we can do, but it's clearly thousands  
4 that led to the 11.

5 JUDGE DYK: Okay. Is that something  
6 that we could have a stipulation about?

7 MR. STONE: I'm certain we could work  
8 out a stipulation at least of the level of this is  
9 what the patent tells us. And we're happy to do  
10 that, Your Honor.

11 JUDGE DYK: And you?

12 MR. PETERSON: Your Honor, I suspect  
13 that we'll be able to work that out at least.

14 JUDGE DYK: Okay.

15 MR. STONE: And then, Your Honor, I have  
16 been unclear about something scientific that I've  
17 heard Your Honor refer to a couple of times. And  
18 so, before I jump into my rebuttal points, I just  
19 want to make one thing clear. It's not that the  
20 light chain of Emicizumab binds factor nine.

21 If you look at any of the diagrams of an  
22 antibody, there's a factor nine heavy chain.

Page 80

1 There's a factor 10 heavy chain. And then both  
2 light chains bind both nine and ten. So it's not  
3 like it's a light on one and heavy on the other.  
4 There's a nine heavy chain. There's a 10 heavy  
5 chain. And on both sides the light chain binds  
6 both nine and ten. I just wanted to clarify that.

7 JUDGE DYK: Okay.

8 MR. STONE: And I would point out in  
9 that since I'm in the neighborhood. There's  
10 nothing in the patent that talks about a light  
11 chain binding more than antibody.

12 I'd like to know -- ask the Court, if  
13 you would, to turn to slide 20 in our slide deck.  
14 I think this might be helpful. I was keeping  
15 track as Mr. Peterson and you, Your Honor, went  
16 through the claims. We have attempted on this  
17 slide to identify within each claim of which types  
18 there are no examples in the patent. And so, I  
19 don't know whether that's helpful to the Court or  
20 not, but I will represent that I didn't hear  
21 anything in the argument inconsistent with this  
22 graphic so that maybe helpful.

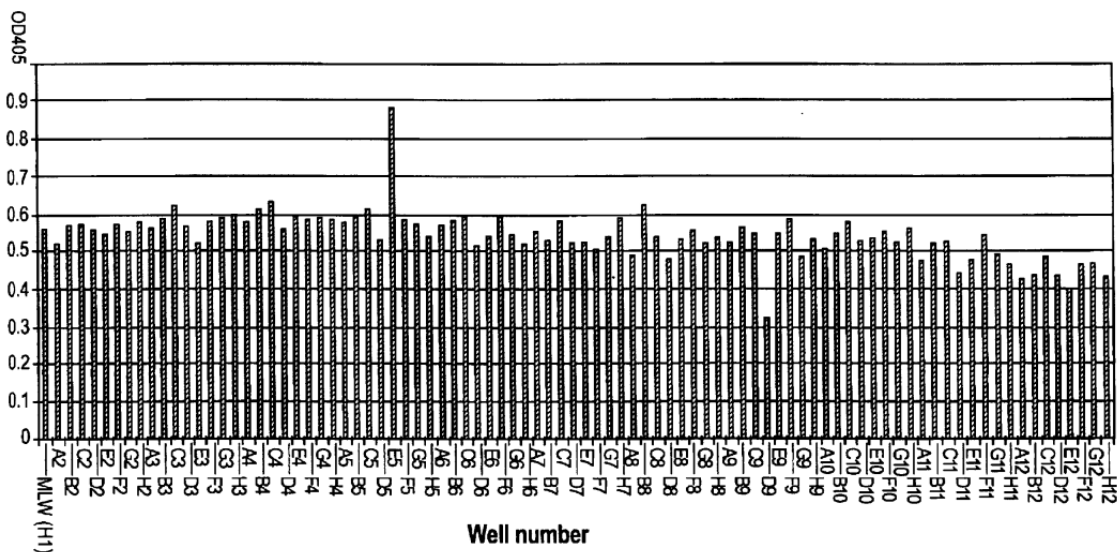


3. To generate the B-cells required to produce a hybridoma, the first step is to inject (or “immunize”) an animal, e.g., a mouse, with the proposed target (or the “antigen of interest”). The antigen is injected, in some cases several times over several weeks through booster injections, to induce an immune response within the animal. The animal’s immune system recognizes the injected antigen as “foreign,” and makes B-cells that produce antibodies that recognize and bind to that antigen. Each such antibody is produced by a B-cell within the animal, and each B-cell produces only a single antibody.
4. The animals are then euthanized, and their B-cells are extracted from their spleens.
5. The B-cells are then fused to myeloma cells to produce hybridomas. The cells are grown in a specially formulated medium that causes unfused cells to die off, leaving behind only fused hybridoma cells.

#### **’590 PATENT INVENTORS’ USE OF THE HYBRIDOMA TECHNIQUE**

6. The antibodies produced by the hybridomas that the ’590 Patent describes as deposited with the European Collection of Cell Cultures (“ECACC”) were made using these hybridoma techniques.
7. The inventors started by injecting mice with Factor IX or its activated forms, activated Factor IX $\alpha$  or Factor IX $\alpha\beta$ . *See* ’590 Patent at col. 9, line 66 to col. 10, line 6. After the animals were euthanized, the B-cells were extracted and fused to myeloma cells to produce hybridomas.
8. The inventors performed and disclosed in the ’590 Patent at least four such fusion experiments, which they labeled #193, 195, 196, and 198. *See* ’590 Patent at col. 10, lines 11-13.
9. The hybridoma cells were then tested to confirm that they produced antibodies. The ’590 Patent reports that the inventors screened the resulting antibodies using a commercially available test called the COATEST VIII:C/4®, *see* ’590 Patent at col. 10, lines 43-46, which, under the Court’s Claim Construction Order, can be used to screen for the claimed increase in the procoagulant activity of Factor IXa.
10. To screen the antibodies for procoagulant activity, the inventors created a “master plate” for each fusion experiment, dividing the generated hybridoma cells across ten 96-well plates, for a total of 960 wells per fusion experiment. *See* ’590 Patent at col. 11, lines 9-12. Typically, each well contained more than one hybridoma (e.g., three to fifteen different hybridomas). *See* ’590 Patent at col 11, lines 23-28.
11. To expedite the screening process, the inventors tested, together, all of the antibodies produced by all of the hybridomas in a given well, using the COATEST VIII:C/4® assay at the same time. *See* ’590 Patent at col 10, lines 43-46. Where the assay showed no procoagulant activity, the inventors knew that none of the antibodies in that given well had such activity.

12. Where, however, the inventors saw activity in the COATEST VIII:C/4® assay for the antibodies secreted from the hybridoma cell(s) within a given well, the inventors then identified and segregated the specific hybridoma cell or cells within that well that produced the antibody or antibodies with procoagulant activity. They did that by diluting the contents of the well and distributing those contents across the 96 wells of a new plate. They then assayed the contents of each of those new 96 wells, again disregarding those in which no activity was observed and again further subdividing those in which activity was observed across the wells of new 96-well plates. This process is called “subcloning.” See ’590 Patent at col. 11, lines 45-49.
13. The ’590 Patent discloses that within each of the four hybridoma fusion experiments, “wells either exhibiting high FVIII-like activity or substantial FVIII-like activity were subjected to subcloning procedures.” ’590 Patent at col. 11, lines 15-17. The patent further discloses that “[f]rom each fusion experiment, several (5-15) master clones (selected from the master plate) were identified and subjected to subcloning.” *Id.* at col. 12, lines 14-16. The patent states that for the cell lines that were subcloned, “most” were subcloned to monoclonality after three rounds of subcloning. See *id.* at col. 12, lines 16-18. For example, Figure 2 reports “the results derived from the fifth master plate of fusion experiment #193.” *Id.* at col. 11, lines 39-41. The plotted values are slightly above the MLW control for approximately 40% of the wells on that master plate, and the patent discloses that the cell pool in “Position ES [sic: should be E5] was identified as exhibiting FVIII like activity significantly higher than the blank (MLW).” *Id.* at col. 11, lines 42-44.

**FIG. 2**

14. After screening candidate antibodies from the four fusion experiments, the ’590 Patent inventors deposited with the ECACC hybridomas producing, and/or disclosed in the patent

the amino acid sequence of the variable regions of, eleven antibodies that increase the procoagulant activity of Factor IXa.<sup>1</sup>

---

<sup>1</sup> As noted during the Summary Judgment Hearing, Genentech disputes that the '590 Patent discloses even eleven antibodies that bind to Factor IX or IXa and increase the procoagulant activity of Factor IXa, but accepts Baxalta's characterization of the evidence for summary judgment only.

**IN THE UNITED STATES DISTRICT COURT  
FOR THE DISTRICT OF DELAWARE**

BAXALTA INCORPORATED and  
BAXALTA GMBH,

Plaintiffs,

v.

GENENTECH, INC. and CHUGAI  
PHARMACEUTICAL CO., LTD.,

Defendants.

Civil Action No. 17-509-TBD

**NOTICE OF APPEAL**



Plaintiffs Baxalta Incorporated and Baxalta GmbH, in the above-named case, hereby appeal to the United States Court of Appeals for the Federal Circuit the Order granting Genentech Inc.'s Motion for Summary Judgment (ECF No. 574) and accompanying Memorandum Opinion (ECF No. 573), entered in this action on January 13, 2022, and from any and all orders, rulings, findings, and conclusions underlying, relating to, pertinent to, or ancillary to the judgment or that merged into or became part of the judgment.

Dated: February 8, 2022

Respectfully submitted,

/s/ Amy M. Dudash

Michael J. Abernathy  
Jason C. White  
Christopher J. Betti  
Amanda S. Williamson  
Wan-Shon Lo  
Maria E. Doukas  
Karon N. Fowler  
MORGAN, LEWIS & BOCKIUS LLP  
110 North Wacker Drive  
Chicago, IL 60606  
T. 312.324.1000  
mike.abernathy@morganlewis.com  
jason.white@morganlewis.com  
christopher.betti@morganlewis.com  
amanda.williamson@morganlewis.com  
shon.lo@morganlewis.com  
maria.doukas@morganlewis.com  
karon.fowler@morganlewis.com

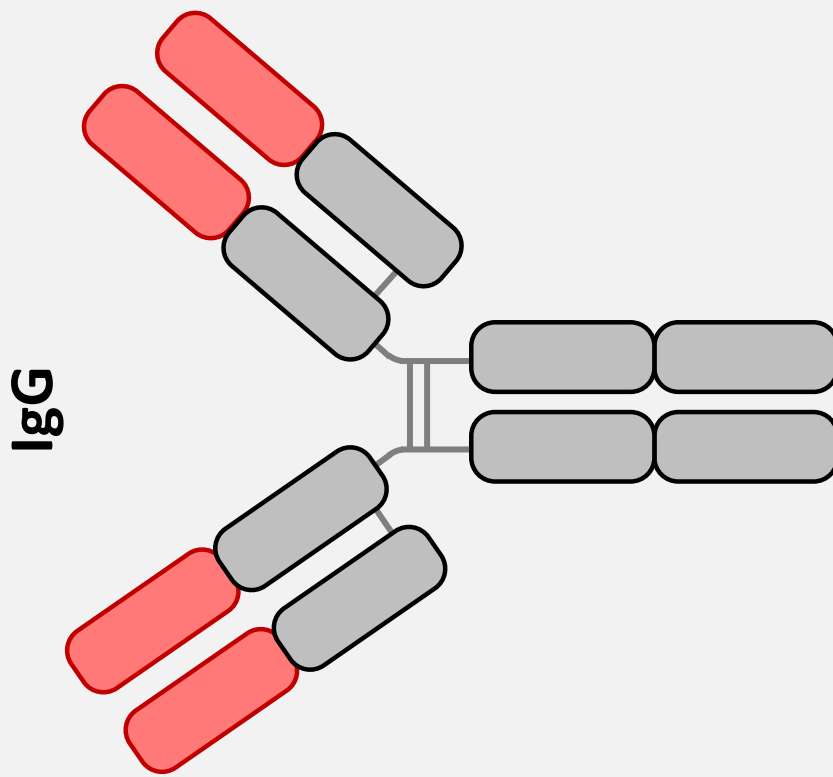
Olga Berson, Ph.D.  
MORGAN, LEWIS & BOCKIUS LLP  
2049 Century Park East, Suite 700  
Los Angeles, CA 90067  
T. 310.907.1000  
olga.berson@morganlewis.com

Jody C. Barillare (#5107)  
Amy M. Dudash (#5741)  
MORGAN, LEWIS & BOCKIUS LLP  
1201 N. Market Street, Suite 2201  
Wilmington, DE 19801  
T. 302.574.3000  
jody.barillare@morganlewis.com  
amy.dudash@morganlewis.com

Margaret A. McGreal  
Julie S. Goldemberg  
MORGAN, LEWIS & BOCKIUS LLP  
1701 Market Street  
Philadelphia, PA 19103  
T. 215.963.5000  
margaret.mcgreal@morganlewis.com  
julie.goldemberg@morganlewis.com

*Attorneys for Plaintiffs Baxalta Incorporated  
and Baxalta GmbH*

## Monoclonal Antibody

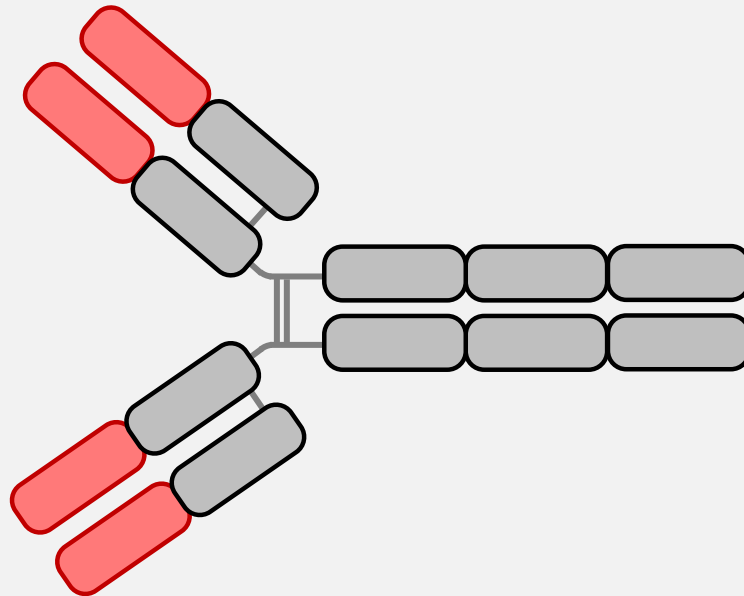


1

## Monoclonal Antibody, Different Isotype

Variable Region

IgE



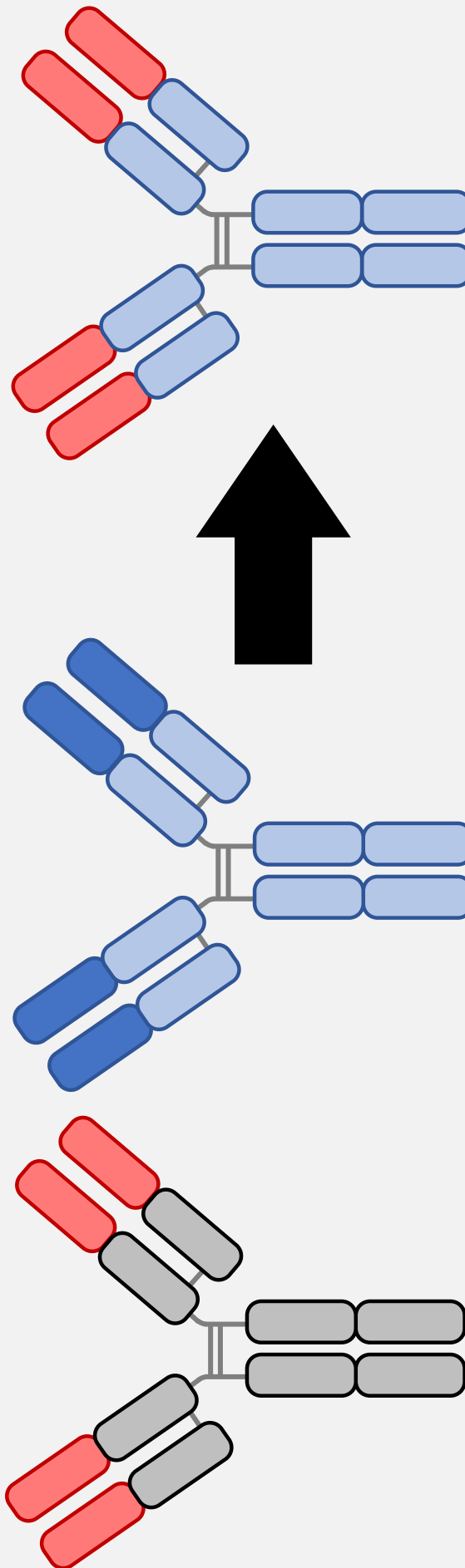
## Chimeric Antibody

Variable Region

Mouse

Human

Chimeric



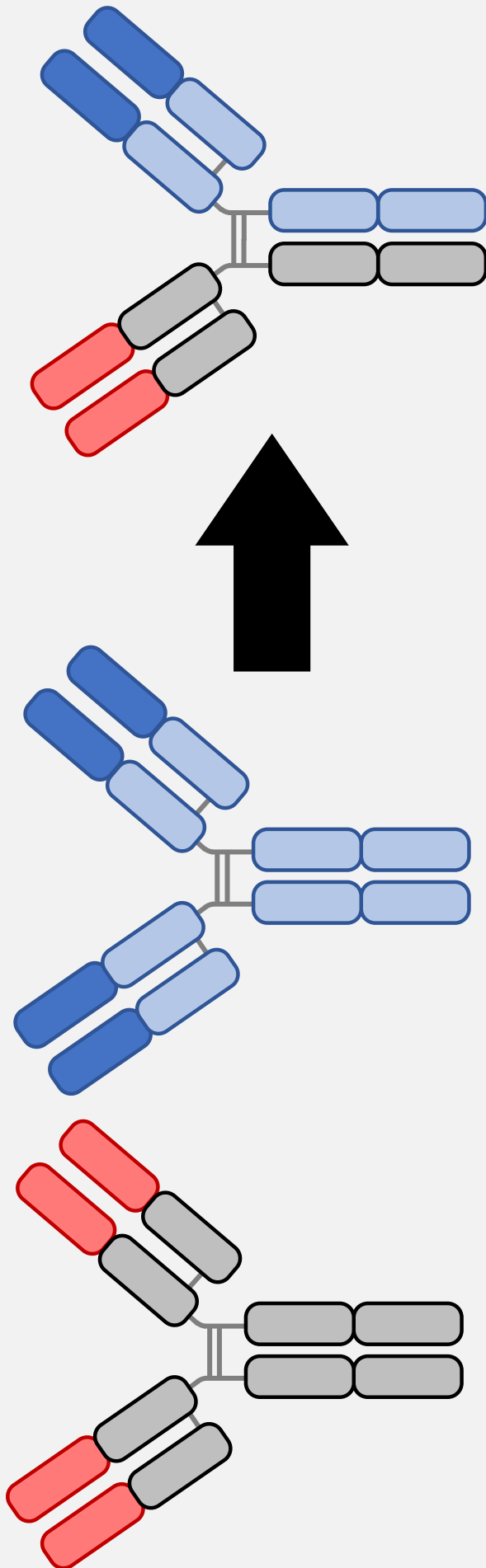
## Bispecific Antibody

Variable Region

**Binds One Antigen**

**Binds Another**

**Binds Both**

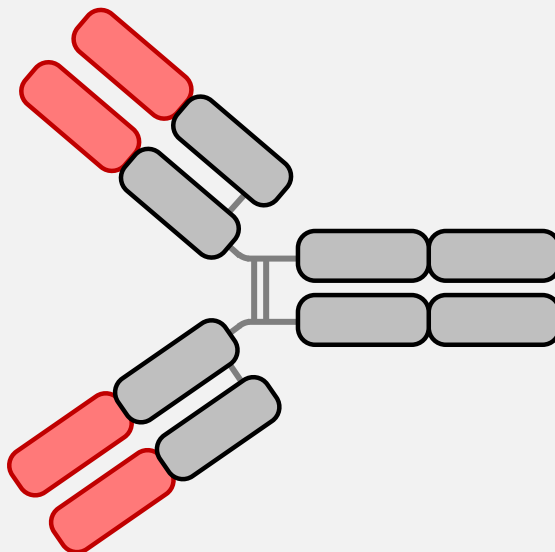
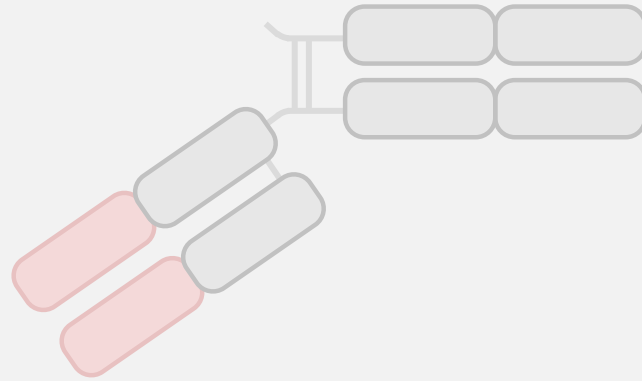
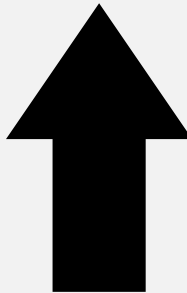


## Antibody Fragments

Variable Region

IgG

Fab



## Antibody Fragments

